JOURNAL OF AGRICULTURAL RESEARCH

VOL. 52 WASHINGTON, D. C., APRIL 1, 1936 No. 7

THE EFFECT OF DIFFERENT COLLOIDAL SOIL MATERIALS ON THE TOXICITY OF CALCIUM ARSENATE TO MILLET[1]

By PHILIP L. GILE

Senior chemist, Soil Chemistry and Physics Research Division, Bureau of Chemistry and Soils, United States Department of Agriculture

INTRODUCTION

At one time it was generally assumed that the arsenical and other poisonous sprays used for controlling insect pests had no injurious effect on soil productivity. This may have been true years ago, when the quantities applied were usually small, but it apparently does not hold for the much heavier applications that are frequently used at the present time. Albert, Cooper, Paden, and others at the South Carolina Agricultural Experiment Station (*1, 2, 3, 7, 8*)[2] have shown, for instance, that the comparatively large applications of calcium arsenate used to control the boll weevil may injure the soil for subsequent crops. According to their observations, sandy soils are more susceptible to injury from such applications than heavier soils and gray soils are more susceptible than red soils. Dratschew (*9*) has shown that a clay may adsorb 1.04 percent of its weight in arsenate ions from a 0.1-normal solution, but he has not studied the effect of such adsorption on plant growth.

It seemed from previous work that the resistance of soils to arsenic injury should be determined by the kind of colloid in the soil as well as by the amount. It also seemed probable that the various effects of the colloids on calcium arsenate would be correlated with the silica-sesquioxide ratios of the materials, since the fixation of phosphate ions is so correlated. Accordingly, experiments were conducted with 36 soils and subsoils to compare the effects of the colloids on the toxicity of calcium arsenate.

METHODS

The method employed was similar to that used in a previous study of the effects of soil colloids on the efficiency of superphosphate (*10*). Foxtail millet (*Setaria italica*) was grown in different soil-sand mixtures, each of which contained sufficient soil to supply 1 percent of colloidal material to the mixture. Increasing applications of calcium arsenate were made to each mixture and the oven-dry weights of the crops determined. By plotting yields against calcium arsenate applications, the quantity of arsenic pentoxide required to reduce the yield by one-half could be determined for each soil-sand mixture. These quantities, minus the quantity of arsenic reducing the yield one-half in pure quartz sand, gave figures for comparing the effects of the different colloids on arsenic toxicity.

[1] Received for publication Oct. 21, 1935; issued April 1936.

[2] Reference is made by number (italic) to Literature Cited, p. 490.

It will be seen from the curves in figure 1 that similar comparative values would have been obtained if the soils or colloids had been compared on the basis of the arsenic required to reduce the yield by one-fourth, three-fourths, or by any specified amount. But the minimum lethal dose could not well be used for comparison, since this value is not sharply defined.

It will be noted that in this experimental procedure the material added to the sand is not the pure colloid but the whole soil in an amount inversely proportional to its colloid content. This procedure, of course, gives a comparison of the colloids only in case the noncolloidal soil material is without effect on the toxicity of calcium arsenate. This assumption is probably substantially correct, since many studies indicate that the reactions of soils to various salts are confined almost exclusively to the colloidal material. Thus, in the following pages when the effect of the soil colloids is discussed

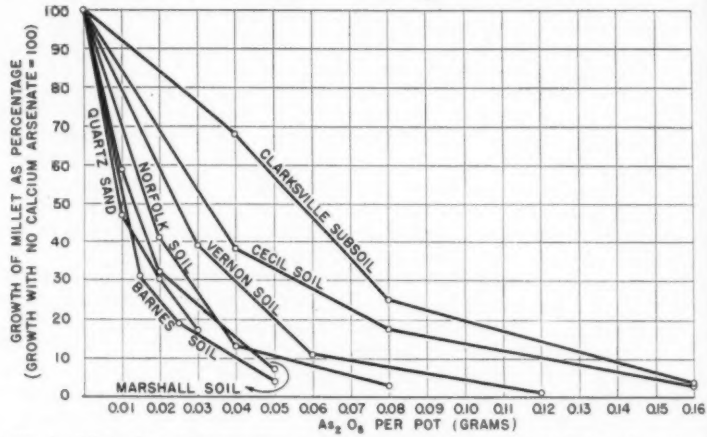

FIGURE 1.—Effects of increasing quantities of calcium arsenate on the growth of millet in soil-quartz sand mixtures.

it is understood that an assumption is involved which seems to be well justified by recent soil investigation.

It may seem that any assumption at all could have been avoided by using the isolated colloidal material. But, aside from its difficulties, this procedure would have led to known errors. Part of the colloid cannot be extracted from certain soils by present methods, and, still more important, the properties of the colloid are altered by the extraction process. Although the alteration may have slight effect on certain laboratory determinations, it may affect plant growth markedly if the freshly extracted colloid is used, as has previously been shown (*10*).

Except for the phosphate application, all pots received the same basic fertilizer; namely, 0.93 g of potassium nitrate, 0.33 g of ammonium sulphate, 0.0185 g of ferric tartrate, 0.42 g of $MgCl_2.6H_2O$, and 0.0015 g of $MnSO_4.2H_2O$ per pot. The phosphate, supplied as superphosphate, varied from 0.2 g of P_2O_5 per pot to 0.6 g of P_2O_5, according to the kind of colloidal material present. In some cases the amount of P_2O_5 necessary for maximum growth could be roughly

calculated from the data of previous experiments; in other cases it was estimated from the silica-sesquioxide ratio of the colloid.

The tricalcium arsenate used was the "c. p. analyzed reagent" of a well-known manufacturer of analytical chemicals. This material and the superphosphate were thoroughly mixed with the sand. The other salts were applied in solution just prior to planting. Glazed earthenware pots holding 5,000 g of quartz sand were used as containers. They were kept in a greenhouse, and their order was shifted every day or two. The moisture content of the sand or sand-soil mixture was maintained at 15 percent. Ten millet plants per pot were grown for 25 to 40 days according to the time of year, ordinarily when the heads were about to appear. The differently treated pots were replicated three times in each experiment.

The soils used in this study were parts of samples utilized in former investigations of soil colloids. Data regarding the samples with laboratory numbers below 500 are given in two bulletins of the United States Department of Agriculture (*11*, *14*), and descriptions of the samples with higher laboratory numbers are given in two others (*6*, *13*). In some cases the colloid content of the soil was determined by adsorption of water vapor, in other cases by mechanical analysis.

CRITICAL ARSENIC VALUES FOR DIFFERENT SOIL COLLOIDS

A few preliminary experiments were conducted to get an approximate idea of the toxicity of calcium arsenate to millet under the experimental conditions. The soil colloids were then compared in 11 experiments. These were conducted at different times of the year under various conditions of temperature and sunlight. Growth varied markedly, of course, in some of the experiments, and it seemed possible that the toxicity of arsenic in the plant might vary with these conditions to such an extent that the results of the different experiments would not be comparable. Such, however, was evidently not the case. Each experiment included a standard series of pots in which calcium arsenate was added to pure quartz sand. Growth in this series varied markedly with the season in which the experiment was conducted, but the quantity of arsenic required to reduce the yield one-half was practically the same for all experiments, irrespective of the season. In 9 of the 11 experiments the critical quantity of arsenic for pure quartz sand varied only between 0.008 and 0.013 g of As_2O_5 per pot, with an average value of 0.011 g having a probable error of ± 0.00036 g. In 1 experiment the critical quantity was not closely determined but was obviously less than 0.016 g, and in the remaining experiments the exceptional value of 0.021 g was obtained.

The results of the quartz sand series provided a blank for the experiment which was to be subtracted from results yielded by the soil-sand mixtures to obtain the effects of the colloids on arsenic toxicity. In view of the constancy of the results of this series it was felt that the average figure was as accurate a blank for any experiment as the individual value obtained for that experiment. Consequently 0.011 g of As_2O_5 was used as a blank for all experiments.

The detailed results of the experiments in which critical arsenic values were obtained for 36 soil colloids are shown in tables 1 and 2. The results of the pure quartz sand series are not reported in these tables, since with the use of the average value for this blank series the individual results are of no interest.

TABLE 1.—*Effects of different soils on the toxicity of calcium arsenate*

[All soils added in sufficient quantity to supply 1 percent colloid]

EXPERIMENT 5 (FEB. 15 TO MAR. 18)

Laboratory no.	Type of soil	Depth	P_2O_5 applied per pot	Calcium arsenate added per pot, expressed as As_2O_5	Oven-dry yield of plants per pot				As_2O_5 required to reduce yield one-half	As_2O_5 reducing yield one-half minus the blank of 0.011 g for quartz sand alone
					Series A	Series B	Series C	Average		
		Inches	*Gram*	*Gram*	*Grams*	*Grams*	*Grams*	*Grams*	*Gram*	*Gram*
10305	Barnes loam	0–9	0.12	0	3.37	3.40	3.83	3.53	0.011	0.000
				.015	1.46	.83	1.35	1.11		
				.025	.55	.56	.94	.68		
				.050	.22	.12	.10	.15		
10341	Miami silt loam	0–1½	.15	0	3.31	3.31	3.28	3.30	.018	.007
				.020	1.74	1.53	1.32	1.53		
				.040	1.02	.63	.85	.83		
				.080	.15	.21	.25	.20		
4439	Davidson loam	0–9	.20	0	3.92	3.11	3.54	3.52	.027	.016
				.04	1.08	.95	.79	.94		
				.08	.32	.29	.30	.30		
				.16	.08	.09	.08	.08		
5891	Nipe clay	0–12	.50	0	1.36	1.33	1.37	1.35	.103	.092
				.15	.30	.37	.39	.35		
				.25	.04	.04	.04	.04		
				.40	.04	.05	.05	.05		

EXPERIMENT 6 (MAR. 24 TO APR. 24)

10082	Carrington loam	0–3	0.15	0	6.45	5.70	5.43	5.89	0.018	0.007
				.01	-------	4.61	3.96	4.28		
				.02	2.95	-------	2.81	2.88		
				.05	.57	.49	.58	.55		
195	Clarksville silt loam	0–10	.20	0	4.73	3.64	3.56	3.98	.032	.021
				.02	3.74	2.58	2.55	2.96		
				.04	1.39	1.27	1.50	1.39		
				.08	.33	.39	.36	.36		
196	do	10–36	.40	0	4.39	6.40	5.72	5.50	.058	.047
				.04	3.84	3.60	3.72	3.72		
				.08	1.59	1.40	1.15	1.38		
				.16	.21	.18	.15	.18		
9415	Cecil clay loam	0–6	.40	0	3.84	4.37	-------	4.11	.031	.020
				.04	1.44	1.60	1.69	1.58		
				.08	.87	.62	.57	.69		
				.16	-------	.13	.14	.14		
6678	Kirvin fine sandy loam	0–12	.35	0	5.63	5.49	5.43	5.52	.056	.045
				.02	4.73	5.10	4.99	4.94		
				.04	3.78	3.74	3.80	3.77		
				.08	1.36	.99	1.43	1.26		

EXPERIMENT 10 (SEPT. 16 TO OCT. 13)

183	Norfolk fine sandy loam	0–8	0.40	0	3.47	3.67	4.26	3.80	0.017	0.006
				.02	1.74	1.63	1.24	1.54		
				.04	.62	.50	.34	.49		
				.08	.10	-------	.11	.11		
184	do	12–36	.50	0	3.50	2.87	2.50	2.96	.034	.023
				.04	1.41	-------	1.03	1.22		
				.08	.34	-------	-------	.34		
				.16	.07	.07	.07	.07		
6718	Vernon fine sandy loam	0–3	.30	0	3.60	3.74	3.50	3.61	.025	.014
				.03	1.24	1.83	1.10	1.39		
				.06	.42	.45	.31	.39		
				.12	.04	.04	.04	.04		
6719	do	3–10	.40	0	5.26	4.85	4.68	4.93	.028	.017
				.04	1.33	1.36	1.26	1.32		
				.08	.23	.22	.27	.24		
				.16	.03	.03	.03	.03		
6720	do	10–27	.50	0	5.13	4.54	4.55	4.74	.033	.022
				.04	1.83	-------	1.89	1.86		
				.08	.42	.61	.43	.49		
				.16	.05	.05	.05	.05		

TABLE 1.—*Effects of different soils on the toxicity of calcium arsenate*—Continued

EXPERIMENT 12 (NOV. 1 TO DEC. 11)

Laboratory no.	Type of soil	Depth	P_2O_5 applied per pot	Calcium arsenate added per pot, expressed as As_2O_5	Oven-dry yield of plants per pot				As_2O_5 required to reduce yield one-half	As_2O_5 reducing yield one-half minus the blank of 0.011 g for quartz sand alone
					Series A	Series B	Series C	Average		
		Inches	*Gram*	*Gram*	*Grams*	*Grams*	*Grams*	*Grams*	*Gram*	*Gram*
B171	Marshall silt loam	0–13	0.20	0	3.40	2.69	2.49	2.86	0.023	0.012
				.01	2.18	2.40	2.37	2.32		
				.02	1.82	1.38	1.85	1.68		
				.03	1.13	.83	.60	.85		
B172	do	13–24	.25	0	2.59	2.77	3.06	2.81	.031	.020
				.01	3.09	2.50	2.59	2.73		
				.02	2.31	1.75	2.18	2.08		
				.03	1.74	1.30	1.40	1.48		
6096	Houston black clay	0–3	.20	0	1.86	1.90	1.14	1.63	.014	.003
				.01	.89	1.00	.98	.96		
				.02	.38	.64	.74	.59		
				.04	.14	.14	.11	.13		
6097	do	14–20	.25	0	2.62	2.54	2.38	2.51	.012	.001
				.01	1.40	1.49	1.28	1.39		
				.02	.60	.66	.57	.61		
				.04	.27	.18	.28	.24		
6098	do	24–36	.30	0	1.89	2.58	2.19	2.22	.014	.003
				.01	1.59	1.77	.95	1.44		
				.02	.77	.55	.67	.66		
				.04	.33	.25	.23	.27		

EXPERIMENT 14 (MAR. 7 TO APR. 6)

299	Chester loam	0–8	0.36	0	5.63	4.85	5.66	5.38	0.046	0.035
				.02	4.17	3.88	4.19	4.08		
				.04	3.09	2.92	3.08	3.03		
				.06	1.98	1.86	1.99	1.94		
300	do	8–32	.50	0	3.18	3.10	3.50	3.26	.053	.042
				.02	3.06	3.10	2.84	3.00		
				.04	2.39	2.31	2.32	2.34		
				.06	1.20	1.44	1.29	1.31		
391	Hagerstown loam	0–8	.36	0	6.12	5.34	5.47	5.64	.079	.068
				.03	4.79	4.60	-------	4.70		
				.06	3.74	3.32	-------	3.53		
181	Ontario loam	0–12	.36	0	4.84	4.91	4.77	4.84	.051	.040
				.02	4.06	3.96	3.91	3.98		
				.04	3.12	2.79	2.63	2.85		
				.06	2.14	2.04	2.09	2.09		
182	do	12–22	.50	0	4.56	4.28	4.18	4.34	.057	.046
				.03	3.40	2.94	2.88	3.07		
				.06	2.01	2.09	2.17	2.09		
				.09	1.31	1.24	1.19	1.25		

EXPERIMENT 15 (APR. 17 TO MAY 12)

297	Manor loam	0–7	0.30	0	4.91	5.49	5.25	5.22	0.031	0.020
				.02	3.52	3.72	3.50	3.58		
				.04	1.87	1.83	1.48	1.73		
				.06	.96	1.13	1.03	1.04		
298	do	7–20	.40	0	4.41	4.71	4.58	4.57	.031	.020
				.02	3.46	3.28	2.93	3.22		
				.04	1.82	1.57	1.39	1.59		
				.06	.93	.90	.83	.89		
10541	Caribou loam	¾–2	.30	0	1.77	1.67	1.96	1.80	.064	.053
				.02	1.88	2.03	1.40	1.77		
				.04	1.30	1.03	1.12	1.15		
				.06	.96	.97	.96	.96		
10543	do	4–6	.40	0	1.03	1.30	2.64	1.66	.051	.040
				.02	1.76	1.45	1.48	1.56		
				.04	1.50	.73	1.04	1.09		
				.06	.72	.39	.61	.57		
305	Stockton clay adobe	0–38	.20	0	6.27	5.77	6.95	6.33	.012	.001
				.01	3.56	3.83	3.32	3.57		
				.02	1.74	1.86	2.01	1.87		
				.04	.37	.58	.49	.48		

TABLE 1.—*Effects of different soils on the toxicity of calcium arsenate*—Continued

EXPERIMENT 77 (AUG. 18 TO SEPT. 12)

Laboratory no.	Type of soil	Depth	P_2O_5 applied per pot	Calcium arsenate added per pot, expressed as As_2O_3	Oven-dry yield of plants per pot				As_2O_3 required to reduce yield one-half	As_2O_3 reducing yield one-half minus the blank of 0.011 g for quartz sand alone
					Series A	Series B	Series C	Average		
		Inches	*Gram*	*Gram*	*Grams*	*Grams*	*Grams*	*Grams*	*Gram*	*Gram*
512	Sharkey clay	0–4	0.20	0	4.60	3.77	4.28	4.22	0.031	0.020
				.01	3.60	3.33	3.34	3.42		
				.02	2.99	3.25	2.13	2.79		
				.04	1.61	1.63	1.54	1.59		
B3632	Houston black clay	1–15	.25	0	4.37	4.54	4.36	4.42	.018	.007
				.01	3.02	3.21	2.84	3.02		
				.02	2.16	1.99	1.85	2.00		
				.04	.48	.56	.57	.54		
189	Wabash silt loam	0–15	.20	0	3.95	4.44	-------	4.20	.026	.015
				.01	3.80	3.55	-------	3.68		
				.03	1.64	-------	-------	1.64		
190	....do	15–36	.20	0	4.27	4.15	3.73	4.05	.024	.013
				.01	3.42	2.78	2.91	3.04		
				.02	1.98	2.38	2.42	2.26		
				.04	.95	1.09	1.08	1.04		
9804	Columbiana clay	0–10	.50	0	3.95	3.75	3.99	3.90	.093	.082
				.02	3.58	3.24	3.72	3.51		
				.04	3.21	3.57	2.99	3.26		
				.08	2.15	2.19	2.44	2.26		

TABLE 2.—*Effects of increasing quantities of soil in soil-sand mixtures on the toxicity of calcium arsenate*

EXPERIMENT 7 (APR. 21 TO MAY 17)

Laboratory no.	Kind of soil mixed with quartz sand and quantity in terms of colloid content per pot	Depth	P_2O_5 applied per pot	Calcium arsenate added per pot, expressed as As_2O_3	Oven-dry yield of plants per pot				As_2O_3 required to reduce yield one-half	As_2O_3 reducing yield one-half minus the blank of 0.011 g for quartz sand alone
					Series A	Series B	Series C	Average		
		Inches	*Gram*	*Gram*	*Grams*	*Grams*	*Grams*	*Grams*	*Gram*	*Gram*
5028	Nacogdoches very fine sandy loam, 25 g	0–8	0.30	0	4.36	4.70	4.01	4.36	0.022	0.011
				.01	3.28	3.54	3.13	3.32		
				.02	2.47	2.20	2.26	2.31		
				.04	.70	.68	.67	.68		
5028	Nacogdoches very fine sand loam, 50 g	0–8	.50	0	5.19	5.27	4.78	5.08	.035	.024
				.02	3.62	3.63	3.25	3.50		
				.04	2.40	2.39	1.57	2.12		
				.08	.49	.47	.34	.43		
5028	....do	0–8	.75	0	5.10	4.90	4.76	4.92	.040	.029
				.02	3.74	3.34	3.43	3.50		
				.04	2.60	2.65	2.19	2.48		
				.08	.72	.71	.95	.79		
5028	Nacogdoches very fine sandy loam, 100 g	0–8	.90	0	5.48	5.73	5.17	5.46	.065	.054
				.04	3.76	3.65	3.46	3.62		
				.08	2.13	2.30	2.11	2.18		
				.16	.29	.43	-------	.36		

TABLE 2.—*Effects of increasing quantities of soil in soil-sand mixtures on the toxicity of calcium arsenate*—Continued

EXPERIMENT 8 (MAY 20 TO JUNE 19)

Laboratory no.	Kind of soil mixed with quartz sand and quantity in terms of colloid content per pot	Depth	P_2O_5 applied per pot	Calcium arsenate added per pot, expressed as As_2O_5	Oven-dry yield of plants per pot				As_2O_5 required to reduce yield one-half	As_2O_5 reducing yield one-half minus the blank of 0.011 g for quartz sand alone
					Series A	Series B	Series C	Average		
		Inches	*Gram*	*Gram*	*Grams*	*Grams*	*Grams*	*Grams*	*Gram*	*Gram*
B171	Marshall silt loam, 25 g	0–13	0.15	0	5.03	4.24	4.39	4.55	0.013	0.002
				.01	3.17	2.41	1.99	2.52		
				.02	1.09	1.64	1.64	1.46		
				.05	.18	.25	.22	.22		
B171	Marshall silt loam, 50 g	0–13	.15	0	4.99	4.28	4.17	4.48	.013	.002
				.01	3.56	2.07	2.05	2.56		
				.02	.88	1.85	1.28	1.34		
				.05	.29	.16	.40	.28		
B171	do	0–13	.225	0	4.69	4.47	3.91	4.36		
				.02	.94	1.84	1.42	1.40		
B171	Marshall silt loam, 150 g	0–13	.15	0	4.59	3.99	4.20	4.26	.029	.018
				.02	2.69	2.18	3.16	2.68		
				.04	1.53	1.47	1.75	1.58		
				.08	.56	.37	.66	.53		
B171	do	0–13	.225	0	4.38	4.26	4.32	4.32		
				.04	1.42	1.47	1.35	1.41		

EXPERIMENT 9 (AUG. 8 TO SEPT. 2)

6679	Kirvin fine sandy loam, 25 g	12–24	0.30	0	3.85	4.02	4.35	4.07	0.041	0.030
				.02	3.25	2.91	3.41	3.19		
				.04	2.03	2.12	2.02	2.06		
				.08	.44	.46	.39	.43		
6679	Kirvin fine sandy loam, 50 g	12–24	.50	0	4.01	3.76	3.96	3.91	.049	.038
				.04	2.25	2.20	2.12	2.19		
				.08	1.11	1.04	1.36	1.17		
				.16	.25	.20	.19	.21		
6679	Kirvin fine sandy loam, 100 g	12–24	.80	0	3.39	3.37	3.51	3.42	.081	.070
				.06	1.93	2.31	2.19	2.14		
				.12	.92	.96	1.02	.97		
				.24	.19	.23	.18	.20		

EXPERIMENT 13 (DEC. 9 TO JAN. 20)

6719	Vernon fine sandy loam, 25 g	3–10	0.35	0	1.96	1.57	1.75	1.76	0.024	0.013
				.02	1.14	.83	.86	.94		
				.04	.62	.51	.64	.59		
				.08	.26	.30	.24	.27		
6719	Vernon fine sandy loam, 50 g	3–10	.50	0	1.96	1.86	1.88	1.90	.040	.029
				.03	1.11	1.03	1.24	1.13		
				.06	.69	.59	.71	.66		
				.12	.16	.24	.21	.20		
6719	Vernon fine sandy loam, 100 g	3–10	.85	0	1.99	1.97	1.84	1.93	.055	.044
				.04	1.11	1.04	1.22	1.12		
				.08	.63	.81	.78	.74		
				.16	.24	.21	.25	.23		

Calcium arsenate is obviously highly toxic to millet in sand and in these soil-sand mixtures. The 0.011 g of As_2O_5 application per pot, which reduces the yield one-half in pure quartz sand, is equivalent to about two parts As_2O_5 per million of sand, or only 4 pounds per acre 6 inches.

The seat of arsenic injury is evidently chiefly in the leaves, as the arsenic-poisoned plants in all cases showed a root development that

seemed normal for the size of the plants. When sufficient calcium arsenate was applied to reduce growth markedly the leaves yellowed, became somewhat striated, and tended to wither; but no particular feature could be singled out as especially distinctive of arsenic injury. Millet, however, does not show nutritional disturbances in such characteristic ways as do tobacco and other leafy plants.

Although the critical quantities of arsenic shown in tables 1 and 2 are in all cases small, there is a rather wide range in the effects of the different soil colloids. The Barnes, Stockton, and Houston colloids in 1-percent mixtures have almost no effect in reducing the toxicity of calcium arsenate, the critical values for these colloids being only 0 to 0.007 g greater than the blank. On the other hand, in the Hagerstown, Columbiana, and Nipe soil-sand mixtures the critical arsenic values are 0.068 to 0.092 g greater than the blank. The extreme range in critical quantities of the samples tested is 0.092 g.

The comparative effects of the surface-soil and subsoil colloids on calcium arsenate may be judged from the data given in tables 1 and 2. In 11 instances the subsoil, or B horizon, was tested as well as the topsoil, or A horizon. Three subsoil colloids, those of the Marshall, Clarksville, and Norfolk soils, were from two to four times as effective as the corresponding topsoil colloids in reducing arsenic toxicity; but in the case of eight soils, the Houston, Wabash, Vernon, Kirvin, Ontario, Chester, Manor, and Caribou, there was no appreciable difference between the effects of surface-soil and subsoil colloids. The B_3 horizons of the Houston and Vernon soils were tested, and these had the same effects as the A and B_1 horizons. This general similarity in the effects of the colloids in different horizons on arsenic is unlike the results obtained with phosphate. In the phosphate study 11 of the 12 subsoil colloids were found to depress the efficiency of the phosphate more than the corresponding topsoil colloid.

Differences in the critical arsenic values cannot at this point be attributed solely to the effects of the different colloids, since the quantity of phosphate applied to the different soil-sand mixtures was varied according to the capacity of the colloid for rendering phosphate unavailable. Early in this work it seemed that the arsenic values might be influenced by the quantity of phosphate applied or by the time of application. If the phosphate were allowed to react with the colloid before the arsenate, or if an excess of phosphate were applied, it seemed possible that the capacity of the colloid for fixing anions might be satisfied by phosphate and the fixation of arsenate might be diminished thereby. Two preliminary tests, however, indicated that it made little difference whether the phosphate and arsenate were applied at the same time or whether one was allowed to react with the soil before the other by alternate wetting and drying. Also, an excess of phosphate apparently had no effect on the toxicity of calcium arsenate, for in three tests with the Nacogdoches and Marshall soils (shown in table 2) an increase in the normal phosphate application by one-half did not significantly alter the yields of the check plants or of plants receiving arsenic.

This failure of the phosphate fertilization to affect the toxicity of calcium arsenate is in accord with results obtained by Antoniani and Fonio (*4*), who found that phosphate ions adsorbed by soils were largely displaced by arsenate ions. Activated charcoal also adsorbs

arsenate ions more strongly than phosphate ions, according to Schilow and Lepin (*15*).

Since the toxicity of calcium arsenate is apparently not affected by variation in the phosphate fertilization, differences in the critical arsenic values may be attributed to differences in the effect of the soil colloids. Obviously, therefore, one of the more important factors in the resistance of soils to arsenic injury is the character of the colloidal material present. Another major factor is, of course, the quantity of colloidal material in the soil.

EFFECTS OF INCREASING QUANTITIES OF SOIL COLLOIDS ON THE TOXICITY OF CALCIUM ARSENATE

Four experiments were conducted to determine how the toxicity of calcium arsenate varies with increasing quantities of colloid in the sand-soil mixture. The results of these experiments are shown in

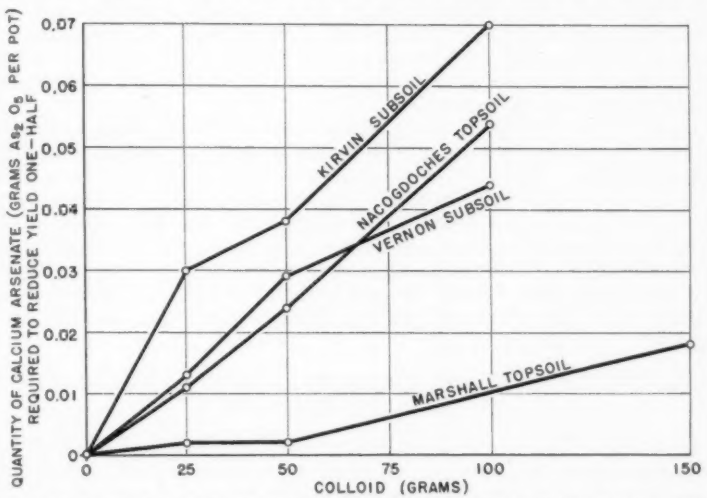

FIGURE 2.—Quantity of calcium arsenate required to reduce yield one-half, as affected by quantity of colloid.

table 2 and figure 2. There are some irregularities in the curves, but in general they approximate straight lines. Deviations are of about the magnitude of the experimental error; hence it appears that the effect of the soil colloidal material in reducing arsenic toxicity increases in approximately direct proportion to the quantity of colloid present. The experimental results upon which this conclusion is based were obtained with soil and sand mixtures containing from one-half to 3 percent of colloidal material. Although the structure of these mixtures varied somewhat, they were all open as compared with structures that might be found in highly colloidal soils. Possibly with mixtures containing from 10 to 30 percent of colloidal material, which would have a closer structure, the proportionality between percentage of colloid and effect on arsenic toxicity would not hold so well.

According to the preceding results, the arsenic resistance of a whole soil would be approximately equal to the percentage of colloid times a figure representing the resistance of the colloid. It is of interest to calculate on this basis the arsenic resistance that whole soils might have. The resulting figures are probably not accurate measures of the arsenic tolerance under field conditions of the soils used in these tests, but they should give a rough idea of the variations in tolerance that might be found in widely different soils.

The figures of tables 1 and 2, representing the quantities of As_2O_5 required to reduce yields one-half, may be used for the specific resistance of 1 percent of colloid. The percentages of colloid in the different soils are given in other publications. The weight of an acre of soil to a depth of 6 inches is taken as 2,000,000 pounds. The pure quartz sand, containing no colloid, would, as already stated, show marked injury with only 4 pounds of As_2O_5 per acre. The Nipe soil, on the other hand, with 60 percent or more colloid, having an exceedingly high resistance, would require 2,112 pounds of As_2O_5 per acre to produce the same degree of injury. An average soil with 20 percent of colloid having an average arsenic value (the mean for colloids tested in this study) would show marked injury from an application of 192 pounds of As_2O_5 per acre.

These figures should be taken as indicating only approximately the order of variation that might be found in soils of the most widely different types. Figures for the arsenic resistances of the whole soils used in this work have not been tabulated because further work would be needed to establish their reliability for field conditions. Probably some factor would be needed to convert values obtained in pot experiments to values applicable to field crops. In pot cultures root growth is altered more or less, and under field conditions there are the modifying effects of leaching, biological activity, and fluctuations in soil moisture.

CHARACTERISTICS OF SOIL COLLOIDS AFFECTING THE TOXICITY OF CALCIUM ARSENATE

Since the soil colloids tested in this work covered a wide range in properties, some evidence can be obtained from the data as to what characteristics of the colloids are responsible for the effects on calcium arsenate.

Apparently the effect of the colloid on calcium arsenate was not connected with acidity in any important degree. The different colloids had acidities ranging from pH 4.2 to pH 8.2, but the arsenic values showed no correspondence to these variations. For example, two soil-sand mixtures of pH 8.1 and pH 4.7, respectively, had approximately the same effect on calcium arsenate, and two other soils, both of pH 4.7, had arsenic values of 0.015 and 0.042. In view of these results acidity could be only a minor factor in arsenic toxicity.

That the effects of the colloids on calcium arsenate are connected with the chemical composition of the materials becomes evident on correlating the arsenic values with features of the ultimate analysis. Table 3 shows that the molecular ratio of silica to sesquioxides, the molecular ratio of iron to silica plus alumina, and the simple percentage of iron all have a significant correspondence with the arsenic values. Other figures of the ultimate analysis of the colloid that were

tried showed considerably less correspondence with arsenic values than the three given in table 3, and a closer correlation with other ratios of constituents is not to be expected after inspecting the figures shown in this table. The marked exceptions to the three significant correlations are the colloids of the Norfolk, Davidson, and Caribou surface soils (nos. 183, 4439, and 10541). It is hard to conceive of a combination of the analytical figures which would bring these colloids into agreement with the arsenic values without throwing the other colloids into marked disagreement.

TABLE 3.—*Correspondence between arsenic values of soils and the chemical compositions of the soil colloids*

Laboratory no.	Kind of soil	Depth	Arsenic value	Molecular ratio of SiO_2 to $Al_2O_3+Fe_2O_3$ of colloidal material	Percentage of iron (Fe_2O_3) of colloidal material	Molecular ratio of Fe_2O_3 to $SiO_2+Al_2O_3$ of colloidal material
		Inches				
10305	Barnes loam	0- 9	0.000	3.30	9.97	0.065
6097	Houston black clay	14-20	.001	3.24	5.92	.043
305	Stockton clay adobe	0-38	.001	2.87	10.57	.063
6096	Houston black clay	0- 3	.003	3.26	5.92	.041
6098	do	24-36	.003	3.25	5.95	.042
183	Norfolk fine sandy loam	0- 8	.006	1.67	11.25	.073
B3632	Houston black clay	1-15	.007	3.26	5.92	.041
B171	Marshall silt loam	0-13	.007	2.91	8.94	.057
10082	Carrington loam	0- 3	.007	2.75	7.75	.049
10341	Miami silt loam	0- 1½	.007	2.50	7.40	.072
190	Wabash silt loam	15-36	.013	3.33	9.57	.057
6718	Vernon fine sandy loam	0- 3	.014	2.65	9.87	.068
189	Wabash silt loam	0-15	.015	3.16	9.75	.058
4439	Davidson loam	0- 9	.016	1.50	12.40	.088
512	Sharkey clay	0- 4	.020	3.23	8.79	.051
B172	Marshall silt loam	13-24	.020	2.88	10.03	.062
298	Manor loam	7-20	.020	1.81	10.29	.065
297	do	0- 7	.020	1.74	10.33	.068
9415	Cecil clay loam	0- 6	.020	1.27	14.57	.077
195	Clarksville silt loam	0-10	.021	2.18	10.05	.065
6720	Vernon fine sandy loam	10-27	.022	2.34	10.53	.065
6719	do	3-10	.023	2.44	10.70	.068
184	Norfolk fine sandy loam	12-36	.023	1.84	11.28	.071
5028	Nacogdoches very fine sandy loam	0- 8	.029	1.08	28.05	.282
299	Chester loam	0- 8	.035	1.77	15.93	.115
6679	Kirvin fine sandy loam	12-24	.038	1.74	14.37	.094
181	Ontario loam	0-12	.040	2.13	10.84	.079
10543	Caribou loam	4- 6	.040	1.41	17.63	.159
300	Chester loam	8-32	.042	1.79	11.34	.073
6678	Kirvin fine sandy loam	0-12	.045	2.02	14.74	.096
182	Ontario loam	12-22	.046	2.08	15.27	.100
196	Clarksville silt loam	10-36	.047	2.10	11.51	.072
10541	Caribou loam	¾- 2	.053	2.95	4.83	.032
391	Hagerstown loam	0- 8	.068	1.91	9.45	.062
9804	Columbiana clay	0-10	.082	.81	15.35	.133
5891	Nipe clay	0-12	.092	.31	62.51	1.185
Coefficient of correlation of arsenic values with molecular ratio, $\frac{SiO_2}{Al_2O_3+Fe_2O_3}$				−0.68±0.06		
Coefficient of correlation of arsenic values with percentage of iron (Fe_2O_3)					+.59±.07	
Coefficient of correlation of arsenic values with molecular ratio, $\frac{Fe_2O_3}{SiO_2+Al_2O_3}$					+.55±.08	

The correlation coefficients given in table 3, together with their probable errors, show that within the limits of probability the two molecular ratios involving iron and the simple percentage of iron all agree about equally well with the arsenic values. Thus it seems that

iron is the chief constituent of the colloid affecting the toxicity of calcium arsenate. This conclusion is supported also by previous work with artificial inorganic gels. Biltz (*5*) found that silica gel had practically no effect on arsenic acid and that an alumina gel adsorbs much less than an iron gel. The activity of an iron gel, however, diminishes with age, according to Biltz (*5*) and to Lockemann and Lucius (*12*).

If iron is the only constituent of the colloid having an appreciable effect on calcium arsenate it might seem that the correlation between arsenic values and percentage of iron or ratios involving iron should be higher than 0.55 to 0.68. However, if the reactivity of the iron in the different colloids varies and does not vary in proportion to the total quantity of iron, a closer correlation could hardly be expected. No data are available on the reactivity of the iron in different colloids, but presumably it is more or less variable. One would expect the iron to be especially reactive in Podzol soils, for instance, since the transfer of iron from the surface stratum to lower layers is especially active in this group of soils. The Caribou, the only true Podzol tested, had an especially high arsenic value for its iron content.

Without attempting to follow the mechanism of the reaction in detail, it may be considered that the soil colloid reduces the toxicity of calcium arsenate by forming an insoluble ferric arsenate with any arsenate ions going into solution. This may be the essence of the matter; but the process is presumably very complicated in detail, several phases being involved—the plant root, the soil colloid, water, and particles of calcium arsenate which are insoluble. Correspondingly, the so-called "arsenic values" may be regarded as representing the quantities of arsenic combined with the colloids in a form so insoluble as to be unavailable to the plant; whereas the actual state of affairs may be somewhat different.

If the alumina of the colloid has no effect on the toxicity of calcium arsenate, it would seem that both correlations of arsenic values with percentages of iron and with ratios of iron to silica plus alumina should be higher than the correlation with ratios of silica to sesquioxides, since this last ratio includes alumina in the same term with iron. But, as a matter of fact, the silica-sesquioxide correlation is at least equal to, if not slightly closer than, the other two correlations. This may be due to the fact that not only the quantity of iron but apparently also the condition of the iron varies approximately with the silica-sesquioxide ratio. At all events, after organic matter is destroyed by hydrogen peroxide most colloids having high silica-sesquioxide ratios are gray, whereas most of those having low ratios are yellow or red. It is possible, therefore, that the silica-sesquioxide ratio evaluates roughly both quantity and reactivity of the iron.

EFFECTS OF SOIL COLLOIDS ON CALCIUM ARSENATE COMPARED WITH EFFECTS ON SUPERPHOSPHATE

Some of the ideas that have been offered regarding the behavior of soil colloids toward arsenate and phosphate are supported by the comparative quantities of arsenate and phosphate rendered unavailable. These ideas, to recapitulate briefly, are that reaction of the colloid with arsenate is dependent only on the iron, whereas reaction with phosphate is dependent on the phosphate content of the colloid

and on both iron and alumina. A previous publication (*10*) gives the quantities of phosphate fixed by a number of soil colloids, some of which were used in this work.

That the phosphate content of the colloid diminishes fixation of phosphate but does not affect fixation of arsenate is in accord with the comparative effects of topsoil and subsoil colloids on the two substances. It was found in the superphosphate study that nearly all topsoil colloids fix less added phosphate than the corresponding subsoil colloids. This was explained on the basis that the topsoil colloid in nearly all cases is higher in phosphorus than the subsoil colloid and is therefore more nearly saturated with phosphate. Granting that this idea is correct, it would follow that the phosphate content of the colloid has little or no effect on arsenate adsorption, since, as previously mentioned, the topsoil and subsoil colloids in most cases fix equal quantities of arsenate.

The conclusion that arsenate fixation is dependent on the iron of the colloid, while phosphate fixation is dependent on both alumina and iron is supported by certain correlations. In the first place, there is a significant but not close correlation between the quantities of arsenate and of phosphate fixed by a number of soil colloids. The correlation coefficient between arsenate and phosphate values is 0.54 for 13 surface-soil colloids and 0.69 for 6 subsoil colloids. Coefficients of this magnitude are about what one would expect if one series of values were dependent on iron alone and the other series on both iron and alumina. The phosphate content of the colloid as a factor affecting fixation of phosphate was largely eliminated by correlating the phosphate values of surface-soil colloids and subsoil colloids separately with the arsenate values.

Further correlations furnish more direct evidence that the arsenate values are dependent on iron alone and that phosphate values are dependent on both iron and alumina. The arsenate values show a fair correlation with iron alone or with iron plus alumina but practically no correspondence with alumina alone. The correlation coefficients are 0.59 for iron, 0.68 for iron plus alumina, and only 0.20 for alumina. Phosphate values, on the other hand, show a fair correspondence with both iron and alumina alone and a fairly close correspondence with iron plus alumina. Correlation coefficients obtained on comparing phosphate values with the chemical constituents of topsoil colloids and of subsoil colloids, separately, were respectively: 0.42 and 0.50 with iron alone, 0.55 and 0.69 with alumina alone, and 0.71 and 0.79 with iron plus alumina.

SUMMARY

This investigation deals with the comparative effects of different soil colloids on the toxicity of calcium arsenate to millet.

Effects of the colloids are determined from comparative growths made by millet (*Setaria italica*) in pot cultures when increasing quantities of calcium arsenate are added to pure quartz sand and to soil-sand mixtures. The soil-sand mixtures contain sufficient soil to supply 1 percent of colloidal soil material. The quantity of calcium arsenate (expressed as As_2O_5) required to reduce the yield one-half in the soil-sand mixture minus the quantity required to reduce the yield

one-half in pure quartz sand is taken as the measure of the effect of the colloid and is called the "arsenic value" of the colloid.

The arsenic values of 36 soil colloids tested show a wide range of variation; namely, from 0.000 to 0.092 g of As_2O_5 per pot. In other words, in some soil-sand mixtures the quantity of calcium arsenate reducing the yield one-half is the same as in pure quartz sand (0.011 g of As_2O_5 per pot); whereas in another soil-sand mixture the quantity of arsenate reducing the yield one-half is 0.092 g of As_2O_5 more than the 0.011 g of As_2O_5 required in quartz sand.

The effect of the soil colloid in reducing toxicity of calcium arsenate increases in approximately direct proportion to the quantity of colloid, at least up to a few percent of colloid. The resistance of a soil to calcium arsenate injury, therefore, depends on the quantity of colloid present and on the specific resistance of the colloid.

On the basis of these pot experiments it appears that whole soils could show resistances to calcium arsenate injury as variable as the following: Soils that are made up of practically pure quartz sand, 4 pounds of As_2O_5 per acre; soils with 20 percent of colloid of average arsenic resistance, 192 pounds of As_2O_5; and soils containing 60 percent of colloid of exceptionally high specific resistance, 2,112 pounds of As_2O_5. These figures may have no practical significance except as indicating possible degrees of variation for certain plants.

The effects of the colloid on calcium arsenate appears to be uninfluenced by the phosphate fertilization, by the acidity of the colloid, and by the presence of calcium carbonate, but it does appear to be influenced by the chemical composition of the colloid. The arsenic values of the colloids correspond roughly with the silica-sesquioxide ratios of the colloids, with the simple percentage of iron, and with the ratio of iron to silica plus alumina: the coefficients of correlation with these features of the chemical composition are, respectively, -0.68 ± 0.06, $+0.59 \pm 0.07$, and $+0.55 \pm 0.08$. It is concluded that the effect of the colloid is dependent on the quantity and reactivity of the iron present.

A comparison of the arsenic values with figures previously obtained for phosphate rendered unavailable by soil colloids tends to support the conclusion that the effect of the colloid on calcium arsenate is dependent only on the iron, whereas the effect on superphosphate is dependent on the phosphate, iron, and alumina contents of the colloid.

LITERATURE CITED

(1) Albert, W. B.
1932. Arsenic toxicity in soils. S. C. Agr. Expt. Sta. Ann. Rept. 45: 44–46, illus.

(2) ——— and Arndt, C. H.
1931. The concentration of soluble arsenic as an index of arsenic toxicity to plants. S. C. Agr. Expt. Sta. Ann. Rept. 44: 47–48, illus.

(3) ——— and Paden, W. R.
1931. Calcium arsenate and unproductiveness in certain soils. Science (n. s.) 73: 622.

(4) Antoniani, C., and Fonio, G.
1929. Ricerche sullo scambio dell' acido fosforico del terreno con acido arsenico. Atti R. Acad. Naz. Lincei, Rend. Cl. Sci. Fis., Mat. e Nat. (6) 9: 344–350, illus.

(5) Biltz, W.
1904. ueber die einwirkung arseniger säure auf frisch gefälltes eisenhydroxyd. Ber. Deut. Chem. Gesell. 37: 3138–3150, illus.
(6) Byers, H. G., Alexander, L. T., and Holmes, R. S.
1935. the composition and constitution of the colloids of certain of the great groups of soils. U. S. Dept. Agr. Tech. Bull. 484, 39 pp.
(7) Cooper, H. P., Paden, W. R., Hall, E. E., Albert, W. B., Rogers, W. B., and Riley, J. A.
1931. effect of calcium arsenate on the productivity of certain soil types. S. C. Agr. Expt. Sta. Ann. Rept. 44: 28–36, illus.
(8) ——— Paden, W. R., Hall, E. E., Albert, W. B., Rogers, W. B., and Riley, J. A.
1932. soils differ markedly in their response to additions of calcium arsenate. S. C. Agr. Expt. Sta. Ann. Rept. 45: 23–27, illus.
(9) Dratschew, S. M.
1933. die adsorption des arsenitions (AsO'_3'') durch die böden. Ztschr. Pflanzenernähr. u. Düngung. Bodenk. (A) 30: 156–167, illus.
(10) Gile, P. L.
1933. the effect of different colloidal soil materials on the efficiency of superphosphate. U. S. Dept. Agr. Tech. Bull. 371, 50 pp., illus.
(11) ——— Middleton, H. E., Robinson, W. O., Fry, W. H., and Anderson, M. S.
1924. estimation of colloidal material in soils by adsorption. U. S. Dept. Agr. Bull. 1193, 42 pp.
(12) Lockemann, G., and Lucius, F.
1913. über die total adsorption von arsen durch eisenhydroxyd. Ztschr. Phys. Chem. 83: 735–758, illus.
(13) Middleton, H. E., Slater, C. S., and Byers, H. G.
1932. physical and chemical characteristics of the soils from the erosion experiment stations. U. S. Dept. Agr. Tech. Bull. 316, 51 pp.
(14) Robinson, W. O., and Holmes, R. S.
1924. the chemical composition of soil colloids. U. S. Dept. Agr. Bull. 1311, 42 pp.
(15) Schilow, N., and Lepin, L.
1920. adsorption als molekularerscheinung. Ztschr. Phys. Chem. 94: 25–71, illus.

THE
DE
IN

By W
mol
Qua

In
(3)[3] i
Newm
soil.
the e
appli
study
perio
were
La
while
in th
feedi
tract,
for t
and u
The
contr
select
exten
but i
field
same
a ma
anese
which
mate
acid
same

Th
guara
other
used

[1] Rece
[2] The
assistan
[3] Refe
[4] Anal
of Chem

Journal
Washin

THE EFFECTIVENESS OF VARIOUS ARSENICALS IN DESTROYING LARVAE OF THE JAPANESE BEETLE IN SASSAFRAS SANDY LOAM [1]

By WALTER E. FLEMING, *entomologist*, and FRANCIS E. BAKER, *assistant entomologist, Division of Fruit Insect Investigations, Bureau of Entomology and Plant Quarantine, United States Department of Agriculture* [2]

INTRODUCTION

In a previous contribution from the Japanese beetle laboratory (*3*)[3] it was shown that larvae of the Japanese beetle (*Popillia japonica* Newm.) were killed by the introduction of certain arsenicals into the soil. Subsequent investigations have been carried on to determine the effectiveness of different arsenicals for this purpose, when freshly applied and after having been in the soil for several years. In the study of the insecticidal action in Sassafras sandy loam during the period 1929 to 1935, which is reported in this paper, over 18,000 tests were made, involving more than 90,000 larvae.

Larvae of the Japanese beetle may be killed upon ingesting arsenic while burrowing through poisoned soil or feeding on roots growing in this soil. When they have a lethal quantity, the larvae cease feeding and burrowing, discharge the contents of the alimentary tract, become flabby, and eventually die. The period of time required for the arsenical to kill depends upon its nature and concentration and upon the development, activity, and susceptibility of the larvae. The nature and concentration of the material applied to soil can be controlled, the larvae of a definite stage of development can be selected, and the activity of the insects can be controlled to a large extent by regulating the food, temperature, and moisture in the soil, but it is impossible to obtain, in different fields, or from the same field at different periods of the year, groups of larvae that have the same susceptibility to arsenic. In any study of the effectiveness of a material as a stomach-poison insecticide for the larvae of the Japanese beetle, it is necessary to conduct the tests under conditions in which the larvae are active, and to compare the killing power of the material with that of a standard stomach-poison insecticide, such as acid lead arsenate, under the same conditions on larvae from the same source.

ARSENICALS USED

The arsenic trioxide used in this study was not analyzed but was guaranteed by the manufacturer to be 98 to 100 percent pure. The other arsenicals had the composition [4] shown in table 1. The names used to designate the various samples are those employed by the

[1] Received for publication Oct. 23, 1935; issued April, 1936.
[2] The writers are indebted to F. M. Wadley, of the Bureau of Entomology and Plant Quarantine, for assistance in the statistical treatment of the data.
[3] Reference is made by number (italic) to Literature Cited, p. 503.
[4] Analyses of these materials were made by the Insecticide Division when it was a division of the Bureau of Chemistry and Soils.

manufacturers and do not imply that definite, pure salts were available. In particular, the two calcium arsenates are to be regarded as containing considerable of the compounds named, but in admixture with such quantities of excess lime and calcium carbonate as to make their gross analyses practically identical.

CONDITIONS FOR TESTING STOMACH-POISON INSECTICIDES ON LARVAE

It has been observed that when the temperature of the soil is below 50° F. the larvae of the Japanese beetle are largely inactive and are affected by stomach poisons only to a limited extent. As the temperature of the soil is raised to 85°, the activity of the larvae increases and the period of time required for an insecticide to become effective is progressively decreased. At a temperature of 80° to 85° a stomach poison may be effective within 2 weeks, whereas at 60° from 6 to 8 weeks may be required. Temperatures above 85° appear to retard the activity of the larvae. Activity is also impaired in soils that are excessively dry or wet.

TABLE 1.—*Percentage composition of the arsenates used in this study*

Arsenate	Moisture	Total arsenic oxide	Total arsenious oxide	Water-soluble arsenic oxide	Total base
Aluminum	7.28	33.71	0.69	0.72	39.82 as Al_2O_3.
Barium	.56	26.69	.10	2.38	65.55 as BaO.
Dicalcium	1.05	40.15	(1)	7.00	40.74 as CaO.
Tricalcium	1.31	40.64	.17	.20	40.57 as CaO.
Ferric	2.28	47.64	.16	1.19	33.66 as Fe_2O_3.
Acid lead	.14	32.41	.28	.41	63.13 as PbO.
Basic lead	.32	22.09	.15	.17	72.59 as PbO.
Magnesium	3.79	32.84	.05	.61	39.58 as MgO.
Manganese	1.40	42.97	.00	1.02	48.32 as MnO. 8.65 as CaO.
Zinc	.91	33.07	3.60	.34	57.94 as ZnO.

[1] Trace.

It was decided to test the effectiveness of stomach poisons at a constant temperature of 80° to 85° F. in Sassafras sandy loam about half saturated with water and to supply an abundance of food by sowing grain in the soil. These tests were conducted in large chambers maintained at a relative humidity of 96 to 100 percent at the desired temperature to prevent excessive loss of moisture from the soil, and lighted artificially to promote the growth of the grain. A description of these chambers has been published (*2, p. 124*).

PROCEDURE FOR CONDUCTING A TEST

In every test commercial acid lead arsenate, which was used as a standard insecticide because several years' experience with the material in the field had demonstrated its effectiveness in killing Japanese beetle larvae, was applied to the soil at the rates of 250, 500, 1,000, 1,500, and 2,000 pounds per acre within a few hours before introduction of the larvae. In mixing, 0.735 cubic foot of soil, sufficient to fill twenty 5-inch earthern pots, was first spread in a thin layer on a smooth concrete floor, and then a weighed quantity of the lead

arsenate was scattered uniformly over the soil and mixed with it by raking and shoveling until uniformly dispersed. The quantities of material added to this volume of soil and the equivalent number of pounds per acre when mixed to a depth of 3 inches are as follows:

Grams per 0.735 cubic foot:	*Equivalent pounds per acre to a depth of 3 inches*
7.3	250
14.7	500
29.4	1,000
44.1	1,500
58.8	2,000

After each batch had been mixed, it was placed in twenty 5-inch pots, the drainage holes of which were covered with 16-mesh wire to prevent the subsequent escape of larvae. An equal number of pots were filled with untreated soil for the determination of the natural mortality of the insect. Other materials were then applied in the same manner, or pots of soil that had been treated previously and exposed to weathering in the field were brought in and the soil was carefully examined to remove any larvae that might be present as a result of natural infestation. Grain was sown in each pot.

The pots were plunged to a depth of 2 inches in moist peat in the constant-temperature chamber. After 48 to 72 hours, when the soil was at the proper temperature and the grain had sprouted, five active third-instar larvae were placed in each pot. The most satisfactory procedure for infesting the pots is to make five holes 2 inches deep with a small dibble, place one larva in each hole, and fill the holes with soil. Third-instar larvae were selected because larvae in this stage are most resistant to insecticides; in addition, it is easier to handle larvae of this age without injuring them, and such larvae are more readily seen in soil during the examination than are those of other instars. It was not feasible, because of cannibalism, to place more than five larvae in a 5-inch pot of soil.

Seven days after the larvae had been introduced, the soil was removed from each pot and the numbers of dead and living were recorded. The soil and the living larvae were then replaced in the pot, the dead being discarded. After being left undisturbed for a second 7-day period, the potted soil was reexamined and the condition of the larvae recorded a second time.

At the end of the first 7-day period usually half of the larvae were surviving in soil treated with the standard insecticide, commercial acid lead arsenate, at rates of 1,000 to 1,500 pounds per acre, and at the end of the second period this condition existed with concentrations of 500 to 1,000 pounds. When a material was considerably more effective than the standard insecticide, usually half of the larvae were alive at concentrations between 250 and 1,000 pounds per acre at the end of the first period and few were alive in any concentration at the end of the second period. When a material was considerably less effective than the standard, the point of median survival was found between concentrations of 1,000 and 2,000 pounds at the end of the second period. The materials that were considerably more effective than the standard were therefore compared with the standard at the end of the first 7-day period, and the other materials were compared at the end of the second period.

EFFECTIVENESS OF VARIOUS ARSENICALS

It has been shown (*6*, *7*, *8*) that the closest agreement in tests with insecticides occurs when the 50-percent death point is selected as the criterion. The method used in determining the median points of survival and the coefficient of effectiveness of the different treatments is illustrated in the determination of the effectiveness of aluminum arsenate that had been freshly applied to the soil. The average number of larvae surviving in each concentration of aluminum arsenate and in each concentration of the standard insecticide tested at the same time, their respective checks, and their standard errors [5] are given in table 2.

TABLE 2.—*Average number of larvae surviving from an original of 5 larvae per pot in various concentrations of aluminum arsenate as compared with their survival in the standard insecticide*

Quantity of material (pounds per acre)	Average number of larvae surviving in—	
	Standard insecticide	Aluminum arsenate
0	3.73±0.123	3.73±0.123
500	2.91± .125	2.16± .173
1,000	1.55± .172	1.33± .176
1,500	.77± .105	.65± .127
2,000	.47± .113	.61± .119

Since an average of 1.27 larvae were dead in the pots of untreated soil in this series, it is considered that in each concentration of these materials this number had died from causes other than poisoning, such as injury or disease. One-half the survival in the untreated soil was therefore selected as the median survival point, or where 50 percent were killed by poisoning. The median survival in this case was found to be 1.86 ± 0.161 larvae.[6]

An examination of the data for the standard insecticide shows that the concentration where 50 percent were killed by poisoning is between 500 and 1,000 pounds per acre. If A is the concentration giving a survival, C, just above the median survival, M, and B is the concentration giving a survival, D, just below the median survival, the median effective concentration of the standard, $M.C._s$, can be estimated by substituting the required values in the equation [7]

[5] It should be noted that the standard error, and not the probable error, is used throughout this paper. The standard error of the mean was calculated according to the equation $\sigma_m = \frac{\sigma_x}{\sqrt{N-1}}$, where σ_x is the standard deviation and N is the number of pots.

[6] The standard error of the median survival in the soil containing an insecticide cannot be determined from the error of the survival in untreated soil. It was therefore estimated according to the procedure outlined by Trevan (*8*). The percentage errors of the survivals above and below the median were plotted and the percentage error of the median obtained by interpolation. The error of the median was then calculated from the value of the median.

[7] This equation for the median effective concentration was derived in the following manner: The median concentration can be expressed by the equation

$$M.C._s = A + (B-A)\left[\frac{C \pm c - M \pm m}{C \pm c - D \pm d}\right].$$

As the samples are from different concentrations, however, the standard error of the difference is equal to the square root of the sum of the squares of the errors of the proportions (*4*, *p. 814*), and this equation becomes

$$M.C._s = A + (B-A)\left[\frac{C - M \pm \sqrt{c^2+m^2}}{C - D \pm \sqrt{c^2+d^2}}\right]$$

When these values are substituted in the equation to determine the error of a quotient (communication from Sewall Wright to B. A. Porter, Bureau of Entomology and Plant Quarantine), the equation becomes

$$M.C._s = A + (B-A)\left[\frac{C-M}{C-D} \pm \frac{C-M}{C-D}\sqrt{\frac{c^2+m^2}{(C-M)^2} + \frac{c^2+d^2}{(C-D)^2}}\right].$$

$$M.C._s \pm e = A + (B-A)\left[\frac{C-M}{C-D} \pm \frac{C-M}{C-D}\sqrt{\frac{c^2+m^2}{(C-M)^2}+\frac{c^2+d^2}{(C-D)^2}}\right].$$

A in this case equals 500, B equals 1,000, C equals 2.91, c^2 equals 0.125^2, M equals 1.86, m^2 equals 0.161^2, D equals 1.55, and d^2 equals 0.172^2. The median effective concentration of the standard was found to be 886 ± 96 pounds, and in like manner the median effective concentration of freshly applied aluminum arsenate was found to be 681 ± 156 pounds.

The coefficient of effectiveness of the aluminum arsenate was then determined by the equation

$$\text{Coefficient of effectiveness} = \frac{M.C._s \pm e}{M.C._t \pm e_1} = \frac{M.C._s}{M.C._t} \pm \frac{M.C._s}{M.C._t}\sqrt{\frac{e^2}{(M.C._s)^2}+\frac{e_1^2}{(M.C._t)^2}}$$

where $M.C._s \pm e_1$ is the median effective concentration of the standard insecticide and its error and $M.C._t \pm e$ is the median effective concentration of the aluminum arsenate and its error. The coefficient of effectiveness of freshly applied aluminum arsenate was found to be 1.30 ± 0.329, indicating that when freshly applied this material is 1.30 ± 0.329 times as effective as freshly applied acid lead arsenate in killing the larvae in the soil.

The coefficients of effectiveness of the various arsenicals in killing larvae were determined immediately after application and at intervals up to 60 months after application. The coefficients are given in table 3.

TABLE 3.—*Coefficients of effectiveness of various arsenates*[1] *and arsenic trioxide against larvae of the Japanese beetle after the arsenicals had been in the soil for different periods of time*

Months in the soil	Coefficient of effectiveness				
	Aluminum arsenate	Barium arsenate	Dicalcium arsenate	Tricalcium arsenate	Ferric arsenate
0	1.30±0.329	1.10±0.216	1.80±0.339	1.46±0.310	1.04±0.140
6	.94± .284	1.09± .233	2.44± .599	1.21± .196	.78± .149
12	1.09± .248	.75± .134	1.24± .243	1.15± .216	.72± .142
18	1.00± .279	.70± .326	1.45± .359	1.28± .200	.66± .132
24	.87± .477	.96± .214	1.27± .259	.80± .165	.42± .165
30	.78± .133	.67± .121	.96± .216		.42± .194
31				1.02± .204	
43	.98± .125	.68± .112	.77± .098	.94± .154	
44					.35± .254
48	0	0	0	0	0
60	0	0	0	0	0

Months in the soil	Coefficient of effectiveness				
	Acid lead arsenate	Magnesium arsenate	Manganese arsenate	Zinc arsenate	Arsenic trioxide
0	1.00±0.148	1.89±0.438	1.97±0.449	1.16±0.181	1.85±0.344
6	.98± .159	1.14± .193	.88± .287	.83± .208	2.75± .547
12	.76± .118	1.38± .203	1.33± .277	1.00± .186	2.00± .530
18	.65± .100	.97± .163	1.05± .274	.92± .162	2.03± .427
24	.63± .110	.87± .333	.82± .182	.75± .178	2.39± .768
30	.53± .102				2.36± .426
31		.83± .105	.92± .128	.68± .090	
43					2.77± .431
44	.25± .056	.31± .061	.37± .070	.33± .073	
48	0	0	0	0	1.30± .251
60	0	0	0	0	1.34± .233

[1] Basic lead arsenate was tested as extensively as the other materials, but the survival was so high, even when used at the rate of 16,000 pounds per acre, that it was not possible to determine the median effective concentration. The coefficient of effectiveness was consistently zero.

DISCUSSION OF RESULTS

When the coefficients of effectiveness of the various materials were plotted against the time that the materials had been in the soil, the functional relationship could be expressed by means of a straight line. To determine accurately the position of this line, the constants of the equation of the line were determined mathematically by the method of least squares (*1, pp. 55–61*). The coefficient of correlation (*1, pp. 121, 128*), $\bar{r}_{zy}$, adjusted for the number of cases, the adjusted standard error of estimate (*1, pp. 121, 128*), $\bar{S}_{yz}$, and the coefficient of determination (*1, p. 120*) were then calculated (table 4). The

TABLE 4.—*Statistical measures showing the association between the coefficients of effectiveness of various arsenicals and the time these arsenicals remained in the soil*

Arsenical	Adjusted coefficient of correlation	Coefficient of determination	Standard error of estimate[1]	Arsenical	Adjusted coefficient of correlation	Coefficient of determination	Standard error of estimate[1]
Aluminum arsenate	0.793	0.629	0.284	Acid lead arsenate	0.942	0.887	0.128
Barium arsenate	.855	.731	.213	Magnesium arsenate	.946	.895	.204
Dicalcium arsenate	.870	.757	.301	Manganese arsenate	.866	.750	.318
Tricalcium arsenate	.841	.707	.289	Zinc arsenate	.946	.895	.127
Ferric arsenate	.883	.780	.168	Arsenic trioxide	.109	.012	.321

[1] In terms of the coefficient of effectiveness.

regression line, the standard error of estimate, and the determined coefficients of effectiveness are presented graphically in figure 1.

For the arsenates there was a high correlation between the coefficient of effectiveness and the period of time that the materials had been in the soil, but there appeared to be little such correlation for arsenic trioxide. The coefficient of determination $(\bar{r}_{zy})^2$ is an arbitrary mathematical measure which shows what proportion of the variance in the values of the dependent variable can be explained by the concomitant variation in the values of the independent variable. It was found that almost 90 percent of the variance in the coefficients of acid lead arsenate, magnesium arsenate, and zinc arsenate could be accounted for by the period the materials had been in the soil. More than 60 percent of the variance in the coefficients of the other arsenates could be attributed to this factor. Since this leaves only a relatively small percentage of the variance to be accounted for by all other factors, it appears that the period of time in the soil was the most important factor associated with the coefficient of effectiveness of these materials. As the coefficient of determination of arsenic trioxide was only 1.2 percent, it is evident that other factors were more important than the period of time in modifying its insecticidal action.

The standard error of estimate shows how nearly the estimated values of the coefficients agree with the determined values. As most of the determined values were scattered throughout the zone ± 1 standard error from the regression line, the standard error of estimate may be used as a measure of the consistency of the insecticidal action of a material. Acid lead arsenate appears to be the most dependable of the arsenates for killing larvae in the soil; zinc arsenate and ferric arsenate were very close to acid lead arsenate in the consistency of their insecticidal action. The other arsenicals were more variable. The variation in the results obtained with aluminum

FIGURE 1.—Effectiveness of various arsenicals against larvae of the Japanese beetle after the arsenicals had been in the soil for different periods of time.

arsenate, dicalcium arsenate, tricalcium arsenate, manganese arsenate, and arsenic trioxide was so great as to raise some doubt regarding the dependability of these materials as stomach-poison insecticides in the soil.

Theoretically, when the linear correlation is high the averages derived from several observations for each period should lie along the regression line, and these averages should very nearly coincide with the estimated values. To have the average of the determined values coincide with the estimated value for each period, it would be necessary to make many tests of a stomach poison in a complex medium such as soil. In view of the long period of time and the labor involved in determining the coefficient of a stomach-poison insecticide in soil, it has not been possible, in most cases, to make more than two or three series of determinations at a given period. Therefore, some fluctuation may be expected in the average determined coefficients around the regression line. It is believed that under the circumstances the regression line, which is based on all the determined values, is probably a more accurate average and a better basis of comparison than the determined values at a given period. It was decided, therefore, to use the estimated values as a basis for comparing the insecticidal effectiveness of the different materials in the soil.

The estimated coefficients of effectiveness at 0 months measure the effectiveness of the freshly applied materials. As these coefficients are subject to variation, as are other statistical constants, the standard errors of the coefficients were determined (*1, p. 253; 4, p. 77*) by the equation

$$\text{Standard error of estimated coefficient of effectiveness} = \frac{\overline{S}_{yz}}{\sqrt{N}}.$$

The estimated coefficients were then arranged in order of increasing difference from the coefficient of acid lead arsenate, and the differences between the coefficient of acid lead arsenate and the other arsenicals were determined as given in table 5. It is generally accepted that when a value is two or more times its standard error the value can be considered significant. It is apparent from these data that ferric arsenate is significantly less effective and the other arsenicals are significantly more effective than acid lead arsenate when freshly applied to the soil.

TABLE 5.—*Significance of the difference between the estimated coefficients of effectiveness of the various arsenicals when freshly applied*

Arsenical	Estimated coefficient of effectiveness	Difference between coefficient of effectiveness of acid lead arsenate and that of arsenical indicated	Ratio
Acid lead arsenate	1.01±0.0199		
Ferric arsenate	.90± .0286	0.11±0.0348	3.2:1
Barium arsenate	1.14± .0336	.13± .0390	3.3:1
Zinc arsenate	1.16± .0246	.15± .0316	4.7:1
Aluminum arsenate	1.28± .0449	.27± .0491	5.5:1
Tricalcium arsenate	1.48± .0519	.47± .0555	8.5:1
Manganese arsenate	1.56± .0571	.55± .0605	9.1:1
Magnesium arsenate	1.61± .0366	.60± .0417	14.4:1
Dicalcium arsenate	2.13± .0618	1.12± .0649	17.3:1
Arsenic trioxide	2.36± .0507	1.35± .0545	24.8:1

It was thought that there might be some correlation between the effectiveness of the various arsenicals and the percentage of total or water-soluble arsenic oxide in the materials. Apparently, however, there was no correlation between the coefficient of effectiveness and the percentage of water-soluble arsenic oxide or of arsenious oxide, although there did appear to be some relation between the coefficient of effectiveness and the total arsenic oxide. The coefficient of correlation and the coefficient of determination were calculated by the regular procedure. The coefficient of correlation was found to be 0.672 and the coefficient of determination, 0.452. As 45.2 percent of the variance in the coefficients of effectiveness could be accounted for by the total arsenic oxide content of the materials, it is apparent that the arsenic oxide content has some influence, although other properties of the materials are more important in modifying the insecticidal action.

The coefficient of regression measures the slope of the regression line; it shows the average change in the coefficient of effectiveness each month the material has been in the soil. The variation likely to be present in the coefficient of regression of the different materials was determined (*1, p. 252; 8, p. 352*) by the equation

$$\text{Standard error of } b_{zy}=\frac{\overline{S}_{yz}}{\sigma_z\sqrt{N}}.$$

The coefficients of regression were then arranged in the order of increasing difference from that of acid lead arsenate, and the differences were determined as given in table 6. It was found that magnesium arsenate and dicalcium arsenate decreased in effectiveness more rapidly than did acid lead arsenate, but no significant difference in the rate of change could be demonstrated with the other materials.

TABLE 6.—*Significance of the difference in the change in effectiveness of the arsenicals in the soil*

Arsenical	Coefficient of regression	Difference between coefficient of regression of acid lead arsenate and that of arsenical indicated	Ratio
Acid lead arsenate	0.0179±0.0022		
Barium arsenate	.0178± .0037	0.0001±0.0043	0.02:1
Aluminum arsenate	.0189± .0049	.0010± .0054	.18:1
Zinc arsenate	.0195± .0023	.0016± .0032	.50:1
Ferric arsenate	.0150± .0029	.0020± .0036	.55:1
Tricalcium arsenate	.0228± .0050	.0049± .0055	.89:1
Arsenic trioxide	.0103± .0056	.0076± .0060	1.27:1
Manganese arsenate	.0277± .0055	.0098± .0059	1.66:1
Magnesium arsenate	.0295± .0035	.0116± .0041	2.83:1
Dicalcium arsenate	.0349± .0068	.0170± .0071	2.39:1

As chemical analyses showed that there was little change in the total arsenic content of the soil in the pots during a period of 60 months, the decrease in the effectiveness of the materials in this case cannot be due to removal of the material from the soil by leaching. Basic lead arsenate was found to be ineffective, even at 10 times the concentration of acid lead arsenate. It appears, therefore, that the loss

of effectiveness of these arsenicals in Sassafras sandy loam can be attributed to the slow conversion of the arsenic into a form, probably a complex basic salt, that is not toxic to the insect. In the field the decrease in effectiveness of an arsenical is influenced to some extent by leaching, and under these conditions the change in insecticidal action can be attributed to both factors.

SUMMARY

During the period 1929 to 1935 a study was made of the relative effectiveness of the inorganic arsenates and arsenic trioxide as stomach-poison insecticides for the destruction of the larvae of the Japanese beetle in the soil. The experiments were conducted under conditions in which temperature, moisture, and food in the soil were controlled, with larvae from the same source. More than 18,000 tests, involving 90,000 larvae, were made with aluminum arsenate, barium arsenate, dicalcium and tricalcium arsenates, ferric arsenate, acid lead arsenate, basic lead arsenate, magnesium arsenate, manganese arsenate, zinc arsenate, and arsenic trioxide.

The effectiveness of the materials in Sassafras sandy loam was determined immediately after application and at intervals up to 60 months. The median effective concentration of each material was compared with that of freshly applied acid lead arsenate, which was used as a standard insecticide and tested at the same time, and the results were expressed as the coefficient of insecticidal effectiveness of the standard.

Almost 90 percent of the variance in the coefficients of acid lead arsenate, magnesium arsenate, and zinc arsenate, and more than 60 percent of that of the other arsenates, could be accounted for by the period the materials had been in the soil, but only 1.2 percent of the variance in the coefficient of arsenic trioxide could be attributed to this factor. It is evident, therefore, that the period of time in the soil was the most important factor associated with the effectiveness of the arsenates but that other factors were more important in modifying the insecticidal action of arsenic trioxide.

Acid lead arsenate appeared to be the most dependable arsenical for killing larvae in soil, with zinc arsenate and ferric arsenate almost as consistent in their action. The variation in the results obtained with aluminum arsenate, dicalcium arsenate, tricalcium arsenate, manganese arsenate, and arsenic trioxide was so great as to raise some doubt regarding their dependability as soil insecticides.

Basic lead arsenate was found to be of no value as a soil insecticide. Ferric arsenate was significantly less effective and the other materials were significantly more effective than acid lead arsenate when freshly applied to the soil. There was no correlation between the water-soluble arsenic oxide content or the arsenious oxide content of the materials and their effectiveness. As 45.2 percent of the variance in the coefficients could be accounted for by the total arsenic oxide content of the materials, it is apparent that the arsenic oxide content has some influence, although other properties of the materials are more important in modifying the insecticidal action.

Magnesium arsenate and dicalcium arsenate decreased in effectiveness more rapidly than acid lead arsenate, but no significant difference in the rate of change could be demonstrated with the other materials.

As there was little loss in the total arsenic content of the soil under the experimental conditions during a period of 60 months, the decrease in effectiveness can be attributed to the slow conversion of the arsenic into a form, possibly a complex basic salt, that is not toxic to the insect. In the field the loss in effectiveness can properly be attributed to the removal of the arsenic by leaching and to its conversion to an ineffective form.

LITERATURE CITED

(1) EZEKIEL, M.
1930. METHODS OF CORRELATION ANALYSIS. 427 pp., illus. New York and London.

(2) FLEMING, W. E.
1934. DEVELOPMENT OF A STANDARD CAGE METHOD FOR TESTING THE EFFECTIVENESS OF STOMACH-POISON INSECTICIDES ON THE JAPANESE BEETLE. Jour. Agr. Research 48: 115–130, illus.

(3) LEACH, B. R.
1926. EXPERIMENTS WITH CERTAIN ARSENATES AS SOIL INSECTICIDES. Jour. Agr. Research 33: 1–8.

(4) PEARL, R.
1923. INTRODUCTION TO MEDICAL BIOMETRY AND STATISTICS. 379 pp., illus. Philadelphia and London.

(5) SNEDECOR, G. W.
1934. CALCULATION AND INTERPRETATION OF ANALYSIS OF VARIANCE AND COVARIANCE. 96 pp., illus. Ames, Iowa. (Iowa State Col., Div. Indus. Sci. Monograph 1.)

(6) STRAND, A. L.
1930. MEASURING TOXICITY OF INSECT FUMIGANTS. Indus. and Engin. Chem., Analyt. Ed. 2: 4–8, illus.

(7) TATTERSFIELD, F., and MORRIS, H. M.
1924. AN APPARATUS FOR TESTING THE TOXIC VALUES OF CONTACT INSECTICIDES UNDER CONTROLLED CONDITIONS. Bull. Ent. Research 14: 223–233, illus.

(8) TREVAN, J. W.
1927. THE ERROR OF DETERMINATION OF TOXICITY. Roy. Soc. [London], Proc., Ser. B, 101: 483–514, illus.

(9) YULE, G. U.
1922. AN INTRODUCTION TO THE THEORY OF STATISTICS. Ed. 6, enl., 415 pp., illus. London.

ERRORS OF ROUTINE ANALYSIS FOR PERCENTAGE OF SUCROSE AND APPARENT PURITY COEFFICIENT WITH SUGAR BEETS TAKEN FROM FIELD EXPERIMENTS [1]

By F. R. IMMER, formerly *associate geneticist*,[2] and E. L. LECLERG, *assistant pathologist, Division of Sugar Plant Investigations, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

The extent and source of experimental error, for the determination of percentage of sucrose and apparent purity coefficient of sugar beets grown in experimental trials, are of considerable practical importance. Very little information is available regarding the magnitude of these errors. Generally, the percentage of sucrose and the apparent purity coefficient are determined from a composite sample of pulp rasped from 10, 20, or 30 beets included in the sample. It is important to know the rapidity with which the error decreases as the size of the sample increases. Since this error will be due to a combination of many factors entering into the analysis, it is of interest to determine also, as far as possible, the source of the major portion of the error. With such information available, it is possible to determine the lines along which improvement in technique, leading to a reduction in experimental error, may be sought.

The following study was made to ascertain the errors of routine analysis for the determination of percentage of sucrose and apparent purity coefficient in sugar-beet samples from experimental plots.

MATERIALS AND METHODS

In 1933 seed of the Kleinwanzleben "Normal" strain of sugar beets was planted on May 7, and the beets were harvested October 4 to 6. In 1934 seed from a composite cross of several high-yielding varieties was planted on May 8, and the beets were harvested October 9 to 11.

The seed was planted in rows 20 inches apart and thinned to a single beet per 12 inches of row. The stand was satisfactory both years. All beets adjacent to obvious skips in the row were removed before harvest, and the results were based on the study of beets with normal competition on all sides.

The design of the experiment was the same in both years. The land was divided into five blocks each year. In 1933 each block was made up of 30 rows. In 1934, 4 of the 5 blocks were 40 rows wide while the fifth contained 35 rows. The plots were 66 feet long in both years. Each block was divided into 10 plots of equal size. Six lots consisting of 2 samples each of 10, 20, and 30 beets were taken at random from each plot. Care was used to sample the entire plot adequately.

[1] Received for publication October 12, 1935; issued April 1936. Cooperative investigations by the Division of Sugar Plant Investigations, Bureau of Plant Industry, U. S. Department of Agriculture, and the Divisions of Agronomy and Plant Genetics and Plant Pathology and Botany of the Minnesota Agricultural Experiment Station. Paper no. 1349 of the Journal Series of the Minnesota Agricultural Experiment Station.

[2] Resigned August 1, 1935; now associate professor of agronomy and plant genetics, University of Minnesota.

The samples were brought to the laboratory immediately. The beets were washed and then each beet was split into halves with a motor-driven circular saw. The weight of the beets, percentage of sucrose, and apparent purity coefficient were obtained for both halves of each sample (hereafter called duplicates). The duplicates were, therefore, the 10, 20, or 30 halves taken from the left or the right side of the saw.

The percentage of sucrose, by direct polarization, was determined by the Sachs-Le Docte cold-water digestion method from a composite of pulp from all the beets in each duplicate. An immersion refractometer equipped with a water jacket for temperature control was used to determine the percentage of total solids. The apparent purity coefficient (hereinafter called purity coefficient) was then calculated by dividing the percentage of sucrose by the percentage of total solids.

A crew of six washed and weighed the beets in each sample, split them into halves, rasped the halves for a composite sample of pulp, determined the saccharimeter and refractometer readings, and washed the glasses and filters after use. The persons reading the instruments had had a short period of training before starting on the regular analyses. The saccharimeter readings were made by the same person during both years; otherwise the laboratory crew was different each year. Speed in operation was stressed throughout, the analyses in this study being made at a rate of slightly over 200 sugar and purity analyses each per day of about 7½ working hours.

Two samples of 10, 20, and 30 beets each were selected from each of 10 plots within each of 5 blocks, making 100 samples of each size. When the beets included in each sample were split into halves, 200 duplicate samples resulted, thus permitting 200 readings to be made for each sample size. The entire experiment was duplicated the second year. The data were analyzed by the procedure described by Fisher [3] as the analysis of variance.

Analyses of variance were made, for each of the three sample sizes in each of the 2 years, for weight of beet, percentage of sucrose, and purity coefficient. Analyses of covariance were made, for each sample size, for the three variables in all combinations consisting of groups of two.

EXPERIMENTAL RESULTS

Table 1 gives, for each sample size, the means for weight per beet (in pounds), percentage of sucrose, and purity coefficient for each year and for the average of both years.

TABLE 1.—*Mean weight per beet, percentage of sucrose, and apparent purity coefficient for 10-, 20-, and 30-beet samples in each of 2 years and the average of both years*

MEAN WEIGHT PER BEET (POUNDS)

Year	10-beet sample	20-beet sample	30-beet sample
1933	1.5344	1.5274	1.4492
1934	.9330	.8714	.9235
Average	1.2337	1.1994	1.1864

[3] FISHER, R. A. STATISTICAL METHODS FOR RESEARCH WORKERS. Ed. 5, rev. and enl., 319 pp., illus. Edinburgh and London. 1934.

TABLE 1.—*Mean weight per beet, percentage of sucrose, and apparent purity coefficient for 10-, 20-, and 30-beet samples in each of 2 years and the average of both years*—Continued

MEAN PERCENTAGE OF SUCROSE

Year	10-beet sample	20-beet sample	30-beet sample
1933	16.0075	15.9635	16.0855
1934	16.8235	16.7270	16.7060
Average	16.4155	16.3452	16.3958

MEAN PURITY COEFFICIENT

1933	87.6702	87.2352	87.4514
1934	85.0634	84.8072	85.0843
Average	86.3668	86.0212	86.2678

The average weights per beet for the three sample sizes were approximately on the same level, indicating that errors due to selection of larger or smaller beets for the different samples had been adequately controlled. These averages assure that the method of selecting beets in the field was satisfactory for the laboratory samples. The average percentages of sucrose and purity coefficient were sufficiently similar for both years to meet the needs of this study.

The mean squares from the analyses of variance for 10-, 20-, and 30-beet samples are given in table 2. The mean squares for weight of beets, percentage of sucrose, and purity coefficient are designated as w^2, s^2, and p^2, respectively, and the mean products by ws, wp, and sp.

TABLE 2.—*Mean squares and mean products of weight per beet (in pounds), percentage of sucrose, and purity coefficient for 10-, 20-, and 30-beet samples in 1933 and 1934*

10-BEET SAMPLES

Year	Apportionment of variation	Degrees of freedom [1]	Mean square [2]			Mean product [3]		
			w^2	s^2	p^2	ws	wp	sp
1933	Plots within blocks	45	2.7327	1.3847	13.0693	−0.6102	−0.5054	2.5657
	Samples within plots	50	1.4697	.5046	7.4557	−.1128	.0146	.9847
	Duplicates within samples	100	.1834	.0790	1.8304	−.0168	−.0315	.1585
1934	Plots within blocks	45	1.0995	1.2684	4.3748	−.3604	−.5739	1.3374
	Samples within plots	50	.3974	.1928	1.3781	−.0278	−.1999	.3353
	Duplicates within samples	100	.0443	.0338	1.0795	−.0010	.0093	.1042
Average of 1933 and 1934.	Plots within blocks	90	1.9161	1.3266	8.7220	−.4853	−.5396	1.9516
	Samples within plots	100	.9336	.3487	4.4169	−.0703	−.0926	.6600
	Duplicates within samples	200	.1138	.0564	1.4550	−.0089	−.0111	.1314

20-BEET SAMPLES

1933	Plots within blocks	45	6.3748	0.9976	19.9208	−0.3807	−0.1758	3.3073
	Samples within plots	50	4.5531	.3024	4.0739	−.5786	−.3615	.5763
	Duplicates within samples	100	.4242	.0570	1.4132	−.0056	−.0516	.1141
1934	Plots within blocks	45	1.7490	.7229	4.2522	.0275	.3943	.9005
	Samples within plots	50	2.2546	.3878	1.6592	−.2072	−.1746	.1489
	Duplicates within samples	100	.1630	.0404	1.0005	−.0034	−.0022	.1122
Average of 1933 and 1934.	Plots within blocks	90	4.0619	.8602	12.0865	−.1766	.1092	2.1039
	Samples within plots	100	3.4038	.3451	2.8666	−.3929	−.2680	.3626
	Duplicates within samples	200	.2936	.0487	1.2068	−.0045	−.0269	.1132

[1] The degrees of freedom due to variation between blocks in each analysis are not shown.

[2] The mean squares for weight of beets, percentage of sucrose, and purity coefficient are designated as w^2, s^2, and p^2, respectively.

[3] The mean products of weight × sucrose, weight × purity, and sucrose × purity are designated as ws, wp, and sp, respectively.

TABLE 2.—*Mean squares and mean products of weight per beet (in pounds), percentage of sucrose, and purity coefficient for 10-, 20-, and 30-beet samples in 1933 and 1934*—Continued

30-BEET SAMPLES

Year	Apportionment of variation	Degrees of freedom	Mean square			Mean product		
			w^2	s^2	p^2	ws	wp	sp
1933	Plots within blocks	45	7.2176	0.6807	18.9185	−0.1119	1.3945	2.7406
	Samples within plots	50	5.5288	.4008	6.9691	−.3122	−.6615	.9022
	Duplicates within samples	100	.6376	.0782	1.9378	−.0001	.0973	.2134
1934	Plots within blocks	45	6.5908	.7684	2.1113	−.5597	−.0578	.5797
	Samples within plots	50	2.5640	.1361	1.7488	−.0344	.1993	.1257
	Duplicates within samples	100	.3226	.0333	.7846	−.0199	−.0902	.1067
Average of 1933 and 1934	Plots within blocks	90	6.9042	.7246	10.5149	−.3358	.6684	1.6602
	Samples within plots	100	4.0464	.2684	4.3590	−.1733	−.2311	.5140
	Duplicates within samples	200	.4801	.0558	1.3612	−.0060	.0036	.1600

The mean squares for variability between blocks and for total variability are not given in table 2 since they are not needed in the present study.

The beets were smaller in size in 1934 than in 1933 (table 1), and from the data in table 2 it appears that the variability was correspondingly reduced. The variance for weight of beets between samples from the same plots was less than one-half as great in 1934 as in 1933. The variability in weight between halves of the same samples (duplicates) also was less than one-half as great in 1934 as in 1933. The variability in weight is of interest insofar as the weight of beets has a bearing on the percentage of sucrose and purity coefficient.

The variance between duplicates within samples for percentage of sucrose was less than one-half as great in 1934 as in 1933 for the 10- and 30-beet samples, with a smaller reduction in the 20-beet samples. The average laboratory-error[4] variances for all samples were for 1933, 0.0714; for 1934, 0.0358; and for the 2 years, 0.0536. The corresponding standard errors of a single analysis would be, therefore, 0.2672, 0.1892, and 0.2315. This reduction probably is due to greater precision in the laboratory in 1934, since the variability between duplicates must be due to laboratory errors alone.

As an average of both years, it cannot be said that the variance between duplicates for percentage of sucrose (laboratory-error variance) was different for the 10-, 20-, and 30-beet samples, the variances being 0.0564, 0.0487, and 0.0558, respectively. Since there seems to be no difference between the laboratory variances for 10-, 20,- and 30-beet samples, it may be concluded that inaccuracies due to splitting the beets into halves, rasping, and mixing the larger mass of pulp from the 30-beet samples as compared with the smaller samples were not significantly different in the three sample sizes.

The laboratory-error variance for purity coefficient, like that for percentage of sucrose, was much lower in 1934 than in 1933. The average laboratory-error variances for all samples were for 1933, 1.7271; for 1934, 0.9549; and for the 2 years, 1.3410. The corresponding standard errors of a single analysis were 1.3142, 0.9772, and 1.1580. The average laboratory-error variances for the 2 years were 1.4550, 1.2068,

[4] The term "laboratory error" is used to designate all possible sources of error that can occur in the laboratory.

and 1.3612 for the 10-, 20-, and 30-beet samples, respectively. There was no consistent variation in the variances with increase in sample size.

It is of interest to compare the relative magnitude of the laboratory-error variances for percentage of sucrose and purity coefficient. The average variance for percentage of sucrose for all samples was 0.0536, while that for purity coefficient was 1.3410; hence the variance of purity coefficient was 25 times as great as that for percentage of sucrose. Table 1 shows that the average percentage of sucrose for all samples was 16.3855, while the average purity coefficient was 86.2186, the latter being 5.26 times as great as the former. It is apparent, therefore, that the relative variance (variance divided by the squared mean) was very nearly the same for both percentage of sucrose and purity coefficient. The relative variance for percentage of sucrose was $.0536 \div (16.3855)^2$, or 0.00020, while that for purity coefficient was $1.3410 \div (86.2186)^2$, or 0.00018. The standard errors for laboratory errors in percentage of the mean were 1.41 and 1.34 for percentage of sucrose and purity coefficient, respectively.

The mean products (table 2) for weight of beets and percentage of sucrose are negative, with one exception, and indicate a small negative correlation. The mean products for weight and purity coefficient were more often negative than positive, and very small. However, the mean products of percentage of sucrose and purity coefficient were positive and indicate positive correlation between these two variables. The significance of the correlation coefficients to be derived from the mean products and mean squares will be discussed in greater detail later.

The variance due to variability between duplicates within samples is due to errors in the laboratory method. The variance due to variability between samples within plots is due to laboratory errors plus the true variance between the individual samples from the same plots. The latter is due to genetic and environmental factors affecting the weight, percentage of sucrose, and purity coefficient of the individual beets.

Table 3 brings together data, taken from table 2, on the two variances for percentages of sucrose for the three sample sizes.

TABLE 3.—*Mean squares for percentages of sucrose and for purity coefficient of 10-, 20-, and 30-beet samples taken in 1933 and 1934*

Year	Apportionment of variation	Degrees of freedom	Mean square for percentage of sucrose in samples having indicated number of beets			Mean square for purity coefficient in samples having indicated number of beets		
			10	20	30	10	20	30
1933	Samples within plots	50	0.5046	0.3024	0.4008	7.4557	4.0739	6.9691
	Duplicates within samples	100	.0700	.0570	.0782	1.8304	1.4132	1.9378
1934	Samples within plots	50	.1928	.3878	.1361	1.3781	1.6592	1.7488
	Duplicates within samples	100	.0338	.0404	.0333	1.0795	1.0005	.7846
Average for 1933 and 1934	Samples within plots	100	.3487	.3451	.2684	4.4169	2.8666	4.3590
	Duplicates within samples	200	.0564	.0487	.0558	1.4550	1.2068	1.3612

While the laboratory-error variances for percentage of sucrose were reduced in 1934 as compared with those in 1933, the ratio of the laboratory-error variance to the variance between samples within plots was relatively similar in both years. Consequently, the interpretation of these data will be based on the averages of both years.

In order to estimate the expected variance for a given number of samples from a plot and a given number of laboratory analyses per sample the formula $K=\frac{p}{n}+\frac{m}{Nn}$ may be used,[5] where

K=required variance per plot,
N=number of samples per plot,
n=number of laboratory analyses per sample,
m=variance due to laboratory errors (between duplicates within samples),
p=variance between samples within plots due to genetic or environmental factors; i. e., variance between samples within plots minus variance between duplicates within samples expressed on a sample basis.

Table 4 shows the average values of p and m for 1933 and 1934.

The values of m are taken directly from table 3, and the values of p are computed from the same table. The value of p for the 10-beet samples, for example, is $\frac{0.3487-0.0564}{2}=0.1462$. To convert this variance from the basis of a single laboratory analysis to a sample basis it is necessary to divide by 2, since two analyses were made on each sample. The variance of a single plot is seen to be made up of two components: (1) The laboratory-error variance and (2) the variance due to the true variability between samples. Both contribute to the error of the plot.

TABLE 4.—*Variance p[1] and variance m[2] in percentage of sucrose of 10-, 20-, and 30-beet samples for the 2-year averages for 1933 and 1934*

Apportionment of variance	10-beet samples	20-beet samples	30-beet samples
p	0.1462	0.1482	0.1063
m	.0564	.0487	.0558

[1] p=variance between samples within plots due to genetic or environmental factors; i. e., variance between samples within plots minus variance between duplicates within samples expressed on a sample basis.
[2] m=variance due to laboratory errors (between duplicates within samples).

As pointed out previously, the laboratory-error variances (m) do not seem to vary with the size of the sample. The estimated variances for the true variability (p) between the 10-, 20-, and 30-beet samples were 0.1462, 0.1482, and 0.1063, respectively. These variances would be expected, theoretically, to be inversely proportional to the size of the sample, i. e., 10-, 20-, and 30-beet samples, as the degrees of freedom increase without limit. The values of p for the 20- and 30-beet samples are approximately proportional to the inverse ratio of the number of beets in the sample, but the value of p for the 10-beet samples is lower than expected.

[5] IMMER, F. R. A STUDY OF SAMPLING TECHNIC WITH SUGAR BEETS. Jour. Agr. Research 44: 633-647 1932.

Increasing the number of laboratory analyses per sample or reducing the laboratory error by more careful technique reduces the variance m. Likewise, increasing the number of samples of beets for sucrose analysis from each plot reduces the variance p. The experimental error of the individual plots is made up of both m and p.

If one 20-beet sample is taken from a plot and duplicate sugar analyses are made on the sample, the variance for the plot (using the averages for both years) is

$$K=0.1482+\frac{0.0487}{2}=0.1726$$

If the two 20-beet samples are taken from a plot and only one laboratory analysis is made on each sample, the plot variance is

$$K=\frac{0.1482}{2}+\frac{0.0487}{2}=0.0984$$

Thus it is seen that if the total number of analyses is held constant, increasing the number of samples from a plot decreases the error of the experiment more rapidly than does increasing the number of laboratory analyses per sample. If but one 20-beet sample is taken from each plot and one anlaysis is made, the variance is

$$K=0.1482+0.0487=0.1969$$

Even though the laboratory variance were reduced to zero, the plot variance (K) would still remain at 0.1482, or 75 percent as great as at present. Thus, increasing the precision in the laboratory can affect only 25 percent of the plot variance obtained in this study.

Greater precision in making the laboratory analyses can be expected to reduce the variance m, but will have no effect on the value of p. While the laboratory errors are probably greater than could be obtained by more careful work by a more adequately trained technician, they are relatively small as compared with the large errors due to the variability between the individual beets.

In table 3 are given the variances of purity coefficients for samples within plots and duplicates within samples for the 10-, 20-, and 30-beet samples. The laboratory error as measured by variance between duplicates within samples, for the average of all three sample sizes, was only 55 percent as great in 1934 as in 1933. The variance for percentage of sucrose in 1934 was 61 percent as great as in 1933. Thus it appears that the reduction in the error in purity coefficient followed closely the reduction in the error in percentage of sucrose. It is the reduction in variance between samples within plots which is more striking, the variance in 1934 being only 74 percent as great as that in 1933. Growing conditions in the autumn of 1933 were normal, whereas in 1934 a very severe and protracted drought greatly delayed growth and reduced the size of the beets. It was shown in table 2 that the beets were more uniform in size and in purity coefficient in 1934 than in 1933. Therefore, it seems reasonable to assume that this reduction in variability in purity coefficient probably was associated to a considerable degree with climatic conditions conducive to greater uniformity between beets.

As an average of both years, the variance m (duplicates within samples) was 1.4550, 1.2068, and 1.3612 for the 10-, 20-, and 30-beet samples, respectively. The variance was lowest for the 20-beet samples but this probably was due to error of random sampling. It appears that the laboratory-error variances for purity coefficient did not vary significantly with size of sample. This fact would follow logically after the conclusions drawn for percentage of sucrose.

The values of variance p for the 10-, 20-, and 30-beet samples, as an average of both years, were 1.4810, 0.8299, and 1.4989, respectively. Thus, the estimated true variance between samples (apart from laboratory errors) was the lowest for the 20-beet samples. These values of variance p were essentially the same for the 10- and 30-beet samples, however.

As an average of both years, the ratio of values of the variance p to variance m for percentage of sucrose varied from 2:1 to 3:1 with different sizes of samples. The same ratio for purity coefficient varied from 1:1 to 0.7:1. From this it is apparent that the laboratory errors are of greater importance for purity coefficient than for percentage of sucrose. Therefore, improvements in laboratory technique leading to lower laboratory errors are relatively more important in reducing the experimental error for purity coefficient than for percentage of sucrose. Any reduction in the laboratory error for percentage of sucrose would reduce the error for purity coefficient as well, since the saccharimeter reading is used in the calculation of purity coefficient.

Since the variances p and m, for the 2-year average of the 20-beet samples, were 0.8299 and 1.2068, respectively, a single sample per plot and but one laboratory analysis would give a variance of $K = 0.8299 + 1.2068$, or 2.0367. The laboratory-error variance (1.2068) would be 59 percent of this plot error. In the case of percentage of sucrose, the laboratory-error variance was but 25 percent of the plot variance.

The laboratory errors for purity coefficient were relatively more important in 1934 than in 1933. Using the 20-beet samples as an example, the laboratory-error variances were 52 and 75 percent of the plot variances for a single analysis on a single sample in 1933 and 1934, respectively.

Since the relative variances (variance divided by squared mean) of laboratory error for percentage of sucrose and purity coefficient were very similar and the estimated variances due to the true variability between beets (variance p) were proportionately much greater than variances m for percentage of sucrose than for purity coefficient, it appears clear that the beets varied considerably less in purity coefficient than they did in percentage of sucrose. This was true particularly in 1934.

When the total number of laboratory tests was held constant, making several analyses per sample reduced only variance m whereas analyzing several samples with only one laboratory analysis per sample reduced both variance m and variance p.

From the foregoing it is apparent that it is preferable to analyze several samples from a plot, with only one analysis of each sample, than to make several analyses per sample.

In table 5 are given the coefficients of correlation for (1) weight of beets with percentage of sucrose, (2) weight with purity coefficient, and (3) percentage of sucrose with purity coefficient. The coefficients were calculated directly from the mean products and mean squares of table 2.

TABLE 5.—*Coefficients of correlation for weight and percentage of sucrose, weight, and purity coefficient, and percentage of sucrose and purity coefficient in 10-, 20-, and 30-beet samples taken in 1933 and 1934*

10-BEET SAMPLES

Year	Apportionment of variation	Degrees of freedom	Correlation coefficient of—[1]		
			w and s	w and p	s and p
1933	Plots within blocks	45	−0.3137	−0.0846	0.6031
	Samples within plots	50	−.1310	.0044	.5077
	Duplicates within samples	100	−.1396	−.0544	.4168
1934	Plots within blocks	45	−.3052	−.2617	.5677
	Samples within plots	50	−.1004	.2701	.6505
	Duplicates within samples	100	−.0258	.0425	.5455
Average of 1933 and 1934	Plots within blocks	90	−.3044	−.1320	.5737
	Samples within plots	100	−.1232	−.0456	.5318
	Duplicates within samples	200	−.1110	−.0273	.4587

20-BEET SAMPLES

1933	Plots within blocks	45	−0.1510	−0.0156	0.7419
	Samples within plots	50	−.4931	−.0839	.5195
	Duplicates within samples	100	−.0485	−.0666	.4020
1934	Plots within blocks	45	.0245	.1446	.5136
	Samples within plots	50	−.2216	−.0903	.1856
	Duplicates within samples	100	−.0419	−.0054	.5581
Average of 1933 and 1934	Plots within blocks	90	−.0945	.0156	.6525
	Samples within plots	100	−.3625	−.0858	.3646
	Duplicates within samples	200	−.0376	−.0452	.4669

30-BEET SAMPLES

1933	Plots within blocks	45	−0.0505	0.1193	0.7637
	Samples within plots	50	−.2097	−.1066	.5398
	Duplicates within samples	100	−.0004	.0875	.5482
1934	Plots within blocks	45	−.2487	−.0155	.4551
	Samples within plots	50	−.0582	.0941	.2576
	Duplicates within samples	100	−.1148	−.1793	.6601
Average of 1933 and 1934	Plots within blocks	90	−.1501	.0784	.6015
	Samples within plots	100	−.1663	−.0550	.4752
	Duplicates within samples	200	−.0367	.0045	.5806

[1] w=weight; s=percentage of sucrose; p= purity coefficient.

In the notation previously employed for designating the mean squares and mean products, the coefficient of correlation between weight of beets and percentage of sucrose would be

$$r = \frac{S(ws)}{\sqrt{S(w^2)}\sqrt{S(s^2)}}$$

The correlation coefficient for variability between duplicates within 10-beet samples in 1933, for example, as calculated from table 2, would be

$$\frac{-0.0168}{\sqrt{0.1834}\sqrt{0.0790}} = -0.1396$$

In testing the significance of these correlation coefficients we may use the 5-percent level of significance to test whether the observed coefficients could have arisen by chance from an uncorrelated population.[6] To exceed the 5-percent level of significance the correlation coefficients would need to be greater than the values given below, for the degrees of freedom in table 5.

Degrees of freedom	*Correlation coefficients at 5-percent point*
45	0. 2905
50	. 2757
90	. 2061
100	. 1955
200	. 1384

The correlation coefficients for weight of beets and percentage of sucrose between duplicates within samples were all negative, though nonsignificant. It is clear, therefore, that the slight differences between the weights of the two halves of the split sample of beets (duplicates) did not significantly affect the percentage of sucrose. Thus, splitting of the beets prior to rasping the pulp for analysis was accomplished with sufficient accuracy to prevent this from being a source of detectable error in the sucrose analyses. While an inherent negative correlation between weight of beets and percentage of sucrose between samples within plots is evident, only in one instance, that of the 20-beet samples in 1933, was the correlation coefficient significant. This coefficient made the correlation for the average of both years significant for that comparison. It appears that variability in weight between samples from the same plots was not sufficiently large to affect appreciably the percentage of sucrose.

The purity coefficient was not significantly correlated with weight of beets for any of the comparisons made.

The coefficients of correlation between percentage of sucrose and purity coefficient were all positive. Only 2 coefficients out of 27 calculated failed to reach the 5-percent level of significance, namely, those for samples within plots in 1934 for both the 20- and 30-beet samples.

As an average for both years, the correlation between percentage of sucrose and purity coefficient, for variability between duplicates within samples, was expressed in coefficients of 0.4587, 0.4669, and 0.5806 for the 10-, 20-, and 30-beet samples, respectively. These coefficients are highly significant and it can be concluded that the purity coefficient was closely associated with percentage of sucrose.

Calculating the regression of purity coefficient on percentage of sucrose, for variability between duplicates within samples, it is found that the regression coefficients from the averages of both years were 2.3298, 2.3244, and 2.8674 for the 10-, 20-, and 30-beet samples, respectively. These coefficients were computed from the mean products and mean squares found in the appropriate rows in table 2. From the regression coefficients it can, therefore, be stated that as percentage of sucrose increased by 1 percent the purity coefficient increased by 2.33, 2.32, and 2.87 percent in the 10-, 20-, and 30-beet samples, respectively.

[6] FISHER, R. A. (See footnote 3.)

It is only the portion of the variability in purity cofficient not associated with percentage of sucrose which is of appreciable value. The percentage of the variability in purity coefficient (as measured by sums of squares) which is associated with percentage of sucrose will be given by r^2. Squaring the coefficient of correlation (as an average of both years) between purity coefficient and percentage of sucrose, for variability between duplicates within samples, it may be said that 21, 22, and 34 percent of the sums of squares for purity coefficient, in the 10-, 20-, and 30-beet samples, respectively, was associated with variability in percentage of sucrose. Roughly one-fourth of the variability in purity coefficient between duplicates was dependent on its inherent association with percentage of sucrose, leaving 75 percent independent of variability for percentage of sucrose.

SUMMARY

A study was made of the errors of routine analysis for percentage of sucrose and purity coefficient in sugar beets taken from field experiments. Comparative analyses were made on 10-, 20-, and 30-beet samples in each of 2 years.

The laboratory errors between 10-, 20-, and 30-beet samples did not vary significantly for either percentage of sucrose or purity coefficient.

The laboratory error was a proportionately greater part of the plot error for purity coefficient than for percentage of sucrose. Increasing the number of samples analyzed per plot would reduce the plot variance more rapidly than increasing the number of analyses per sample.

A negative, though nonsignificant, correlation coefficient for variability between duplicate analyses of the same samples was obtained between weight of beets and percentage of sucrose or purity coefficient.

The correlation between percentage of sucrose and purity coefficient was positive and highly significant. As the percentage of sucrose increased by 1 percent the purity coefficient increased by 2.33, 2.32, and 2.87 percent in the 10-, 20-, and 30-beet samples, respectively. Approximately one-fourth of the variability in purity coefficient, as measured by the differences between duplicate samples, could be ascribed to its inherent association with percentage of sucrose.

AVAILABILITY OF THE COPPER OF BORDEAUX MIXTURE RESIDUES AND ITS ABSORPTION BY THE CONIDIA OF SCLEROTINIA FRUCTICOLA[1]

By M. C. GOLDSWORTHY, *associate pathologist*, and E. L. GREEN, *associate biochemist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture*[2]

INTRODUCTION

The present paper deals with the deposit left by the drying of a bordeaux mixture spray and its effect on the conidia of *Sclerotinia fructicola* (Wint.) Rehm. This residue consists of the sediment or precipitate and the materials that were dissolved in the water that evaporated. It has been established by previous workers that a fresh residue usually contains calcium hydroxide, calcium sulphate, calcium carbonate, basic copper sulphate, and copper hydroxide. That it is very slightly soluble, as indicated by its resistance to washing by rains, has been established by studies of the copper compounds it contains.

The work reported herein was chiefly concerned with the toxicity of the residues after aging and weathering, the latter term including the effects of rain, dew, atmospheric gases, and plant excretions.

REVIEW OF LITERATURE

Studies of the physical and chemical properties of bordeaux mixture (*7*, *22*, *24*)[3] have shown that the copper is in the form of basic sulphates, which investigators have assumed become slightly soluble when acted upon by carbon dioxide of the air or by spore and host excretions.

A large literature has been built up around the above-named hypotheses, which have been well discussed by Martin (*21*) and McCallan (*19*). According to these hypotheses, the toxicity of the basic sulphates of copper is due to the liberation of soluble copper after the materials have been deposited upon the host plants, and not to the soluble ions and soluble complexes inherent in the deposits themselves.

Whetzel and McCallan (*29*, *p. 3*) summarized the published information on the solubility of bordeaux mixture in these words:

> It is generally recognized, however, that certain of our standard fungicides, notably bordeaux mixture and sulfur dust, are not sufficiently soluble in pure water to exercise their fungicidal properties.

Although it has been the accepted theory that the precipitates of bordeaux mixture are not in themselves sufficiently soluble to exert a toxic influence, and a considerable amount of research has

[1] Received for publication Nov. 27, 1935; issued April, 1936.
[2] The writers wish to express their appreciation to J. W. Roberts, of this Division, for his helpful suggestions during the course of this investigation, and to other members of the Bureau of Plant Industry and to members of the Bureau of Chemistry and Soils for their criticisms in the preparation of the manuscript.
[3] Reference is made by number (italic) to Literature Cited, p. 532.

been carried out to determine how and why these precipitates become soluble, it is only recently that we have come to an understanding of some of the principles involved. Although it has been recognized by Clark (*10*), Crandall (*11*), Barker and Gimingham (*4*), McCallan (*19*), Lutman (*17*), and many others that bordeaux mixture precipitates are toxic to fungus spores from the time they are applied, it still remains to be proved just how that toxic effect is produced.

The study of bordeaux mixture must take account of its chemical composition and properties, and for this reason a review of the pertinent knowledge on these subjects is presented. The chemical composition of bordeaux mixture is known to be quite complex. One reason for this is found in the wide range of proportions and concentrations of the solution of copper sulphate and the solution or suspension of calcium hydroxide that are stirred together to make bordeaux mixture.

Pickering (*24*) concluded that the proportion of lime should not exceed that necessary to react with all the copper sulphate. He found this to be three-fourths of a chemically equivalent weight, or a trifle more than 1 part by weight of hydrated lime to 5 parts of copper sulphate crystals. This fact and his studies of the precipitate showed that the copper compound formed by the reaction was the basic sulphate $CuSO_4.3Cu(OH)_2$, the same that had been described long before by Proust (*26, p. 34*) as the result of the prolonged reaction of copper hydroxide with copper sulphate. According to Posnjak and Tunell (*25*), extensive studies of copper compounds have by now led most of the interested authorities to conclude that at temperatures between 0° and 40° C. and near the neutral point this compound is the only one formed. Pickering's restriction of the lime kept the alkalinity of his preparation in this same range. An alkalinity greater than 0.05 normal (pH 12.5) cannot be attained with lime because of its small solubility. Thus certain basic compounds of copper that have been shown to be formed in more alkaline solutions are formed only slowly and incompletely in calcium hydroxide solutions.

Injuries to host plants have compelled the use of much greater proportions of lime than those recommended by Pickering (*24*); in fact, the weight of lime taken at present is more likely to be at least equal to the weight of the copper sulphate than to be only a fifth of it. The sediment of these mixtures contains calcium hydroxide in addition to the sulphate, for more is present than will dissolve. This circumstance makes the basicity of the insoluble copper compounds difficult to estimate, and accordingly divergent results have been reported. These results have led to suggestions that a number of basic compounds of copper and double compounds of lime and copper are formed. However, Martin (*22*) has presented evidence of a slow transformation of the basic sulphate first formed to the hydroxide, and offers this as an explanation of the divergent results.

Since the immediate effect of bordeaux mixture in the control of fungi (*4, 10, 11, 17, 19*) and obvious damage to certain host plants (*3*) cannot be adequately explained except by assuming that some of the copper passes into solution, the copper compounds of the residue cannot be considered strictly insoluble. In one of the earliest papers on bordeaux mixture, Millardet and Gayon (*23*) suggest an inherent solubility, and more recently Hockenyos (*14*) and Ribéreau-Gayon

(*27*) found that this solubility measures several parts per million. In the work presented in this paper the writers have found that the copper of bordeaux mixture dissolves in water at the rate of about 4 parts per million. The behavior of bordeaux mixture as reported herein indicates that something different from the inherent solubility of these copper compounds is responsible for the fact that the copper is readily taken up from certain residues by fungus spores. These results agree better with the theory that there is present a varying quantity of a certain sort of copper compound, not necessarily ionic, which is subject to absorption by the spores. For this property the term "availability" is suggested to distinguish it from solubility, which means the quantity of the residue constituent that will dissolve without first undergoing change.

That the solubility of unaltered bordeaux mixture residues in large quantities of water does not yield sufficiently high concentrations of copper to be harmful to the test spores has been proved by a number of investigators (*4*, *19*, *21*, *28*); but contact with the particles of the same residues in small quantities of water has killed some of the same spores with little delay. It is also well known that the shorter the distance between a particle of residue and a spore the sooner toxicity becomes evident (*4*).

The literature discussed so far has dealt exclusively with freshly made precipitates or with fresh residues. It is obvious that practical work in the field has to do with residues in this condition for only a relatively short time and that most of their usefulness comes after a certain period of aging and exposure to weather. The resultant effects upon the spray residues need to be understood, particularly in establishing bases of comparison in the development of new fungicides. Recently Branas and Dulac (*5*) reported the results of their studies on copper dissolved from residues that were washed with solutions made up to imitate rain water; however, the residues were filtered off and were not studied.

MATERIALS AND METHODS

Residues resulting from bordeaux mixture sprayed upon microscope cover slips were exposed to natural weathering in the orchard and then brought into the laboratory for critical tests, both as to chemical changes and as to toxicity to organisms. Tests were also carried out with residues that had been aged in the laboratory.

Conidia of *Sclerotinia fructicola* were used in this work. The quantity of similar work done with this organism attests its suitability. These conidia are large, hyaline, easily stained, and have large vacuoles and well-defined granules. Germinability is high and dependable. For the most part the conidia were from 10- to 16-day-old cultures grown upon nutrient potato hard agar. Occasionally the conidia were obtained from growths upon sterile string bean and from rotting peaches and plums.

The precautions given by McCallan (*18*) for germination tests were strictly observed. Suspensions of the conidia were prepared so that they contained about 30 cells to a low-power field ($\times$ 125).

The tests for toxicity were carried out by placing the conidia of *Sclerotinia fructicola* in drops of water or dilute nutrient solutions upon

the residues, the cover slip being inverted and used as the cover of a Van Tieghem cell. All tests were made in duplicate.

Thick (no. 2) cover slips were used to reduce breakage. The 24- by 50-mm size was chosen because two tests could be made with each slip by breaking it into halves. The slips were cleaned, numbered on the back, and laid upon paper towels on a horizontal surface, none touching another slip. They were then sprayed by means of a hand atomizer until each was covered with a complete layer of spray liquid, and dried. Averaging a number of determinations of total copper (Cu) upon slips prepared in this way gave 36 micrograms per square centimeter, which is in reasonable agreement with the amount found by Hockenyos and Irwin (*15*) and by Holland, Dunbar, and Gilligan (*16*) for the surface of leaves sprayed in the orchard.

The bordeaux mixture spray used in all tests contained the equivalent of 2 pounds of copper sulphate ($CuSO_4.5H_2O$), 4 of hydrated lime (70 percent CaO), and 2 of bentonite to 50 gallons of water. The bentonite was added to improve adhesiveness and was used in other tests being compared with these. For comparison, in order to evaluate the fungicidal effect of the lime, residues from similar sprays where zinc replaced copper were used. The zinc compounds seemed to be without permanent effect upon the germination of the spores, and the alkalinity of the lime would be neutralized in about the same way as in bordeaux mixture. While it may be argued that a calculated quantity of sulphuric acid would be a more defensible check than zinc sulphate, the results seemed to prove the zinc-lime spray satisfactory. Its use was convenient because it gave residues that were toxic only for a day or so and apparently indifferent afterward. This no doubt was due to the loss in the initial alkalinity of the fresh precipitate.

After being dried, the slips were either stored in the laboratory in a microscope-slide box modified to contain them or hung up among the leaves in the orchard in a special holder designed for this purpose (fig. 1). This holder was made by fastening a piece of wood to one

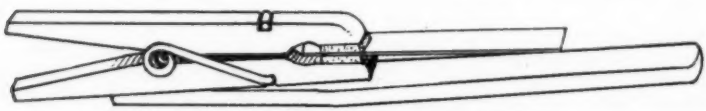

FIGURE 1.—Holder for exposing sprayed slips in orchards.

side of a clamp clothespin to protect the cover slip from breakage by wind. The jaws were surfaced with cork. Unless a means can be found to make a satisfactory germination test upon a leaf, this seems to represent the nearest possible approach to exposing residues to the environmental influences that affect residues upon the plant. Unsprayed slips were sometimes hung in the orchard and were sprayed when the tree was sprayed. Work reported in this paper suggests that studies of this kind offer information not to be had by aging the residues in any other known way.

Initial germination was found to be somewhat more vigorous and constant when nutrient materials were added to distilled water, and such a medium appeared to be more suitable than distilled water alone. Clumping of the conidia, or the tendency of some of the cells to resist

wetting and, therefore, submersion, frequently occurred in most of the dilute nutrient media used. This is a distinct disadvantage, since some of the cells do not have an opportunity to absorb the nutrient media and germination may be delayed. To obviate this, recourse was had to a material in no way injurious in itself, namely, coenzyme R, which has the property of wetting each spore so that the cells have the same electrical charge. Germination was found not to be particularly hastened by this method, but it was noted that a greater number of conidia began their activity at one time. This, no doubt, is a consequence of each cell having the same opportunity of absorbing nutrients. The original paper on coenzyme R, by Allison, Hoover, and Burk (*1*), described it as a respiratory coenzyme. The present work indicates that it also produces a change in the surface tension of the cell walls of the organisms, allowing this surface to become wet with the suspensoid containing the nutrient materials. In this manner the uncertain effects of clumping have been eliminated, and it is felt that the work is more standardized because of it. When the spore suspension was applied to a surface of spray residue, the liquid always showed a strong tendency to spread out among the particles of the spray residue. This, no doubt, was caused by the ability of the suspensoid to wet the particles of spray residue thoroughly. When such a film was placed in the saturated atmosphere of a Van Tieghem cell it remained so thin that germination was interfered with, apparently because of a lack of "free" water. Accordingly, the residue outside of a circle 6 or 7 mm in diameter was scraped away from the cover slip; with this precaution satisfactory germination tests were obtained.

After a small drop of the suspension delivered by a microloop had been transferred to the prepared cover slip containing the residues, the cover slip was inverted over a Van Tieghem cell and incubated at room temperature for a period of time varying according to the object of the test.

After every germination test some of the conidia were transferred from the cover slip to an agar slant to find out whether they had been killed or whether their germination had been merely inhibited. McCallan (*19*) and McCallan and Wilcoxon (*20*) do not mention this step, but it is important for the true evaluation of a fungicide. It is known that toxic materials in less than lethal doses or minor deficiencies in the environment may prevent spores from germinating. But failure of germination alone, without plasmolysis, is not a conclusive proof of death, for if the spore is removed from the unfavorable environment and the toxic material absorbed is given an opportunity to diffuse into a medium containing practically none of it, germination may take place. The implications of such a result in the field use of sprays is obvious. Residues from which spores germinated after such a transfer have been designated "inhibitory."

It was inevitable that the investigator should form an impression of the quantity and tenacity of the residue while scraping off the unwanted portion. This is presented with the data on germination. "Excellent" was used to describe a residue that seemed as heavy as when freshly prepared. Other terms were used with their usual connotations, "fair" meaning a barely continuous film over the glass and "poor" a residue with obvious defects.

A colorimetric method was employed to detect soluble copper on the bordeaux mixture residues and in the solution drops on the residues. During the last 10 years a number of sensitive reagents for copper have been developed (*8, 9, 12, 13*). The one used in this work is the sodium salt of nitroso–1, 8–dihydroxynaphthalene–3, 6–disulphonic acid, discovered by Brenner (*6*), and adapted for the microdetermination of copper by Cherbuliez and Ansbacher (*9*). The concentrated stock solution of this compound, called "the chromotropic reagent" by Ansbacher, Remington, and Culp (*2*), diluted with an equal volume of distilled water, is to be understood in this work where treatment with chromotropic solution is mentioned. Other chemicals scarcely affect the formation of the diagnostic colored compound formed on the addition of this solution to substances containing soluble copper, and the simplicity of the operations required commended it for the present purpose. The color is an intense purple, and because it is different from other colors generally encountered in plant cells, it is useful for the microscopic recognition of copper inside cells.

Direct application of a drop of chromotropic solution to a fresh, highly toxic copper-bearing residue invariably gives the characteristic purple color which is indicative of the soluble copper inherent in the residue. Generally a much deeper color is obtained if the residue is first treated with the fumes of nitric acid, then wet with a drop of 1-percent ammonium hydroxide and allowed to dry. There is reason to believe that this treatment disengages the copper from certain complexes and insoluble compounds in the residue which do not give the test before such treatment. This procedure is accordingly considered to yield a purple "spot", the intensity of which is roughly proportional to the total amount of copper present.

As has been mentioned, each 24- by 50-mm cover slip was broken into two pieces and duplicate germination tests were run. From one of these a transfer was made to an agar slant to see whether viable spores still remained. The other half was allowed to dry and was then treated with nitric acid, ammonia, and chromotropic solution, as described above, and the intensity of the resulting purple spot was recorded. Values assigned to these intensities were as follows: 4, Indistinguishable from a freshly prepared slip; 3, noticeably weaker than 4; 2, having a color roughly half as deep as that of 4; and 1, very faint.

CHANGES IN TOXICITY, APPEARANCE, AND TOTAL COPPER OF BORDEAUX MIXTURE RESIDUES AFTER WEATHERING

Table 1 presents one of a series of tests conducted with the methods just described. The cover slips were sprayed on August 7, 1933. Some were hung up in the fruit-disease orchard at Arlington farm, Rosslyn, Va., and removed for tests after the indicated intervals. Others were stored in a closed box in the laboratory until August 18, when they were placed in holders in the same trees. They were also tested after intervals of exposure. The total number of days of orchard or laboratory exposure and the total rainfall for each slip were recorded. The differences in effect of age without rainfall and of age combined with rainfall are shown.

TABLE 1.—*Changes in toxicity, appearance, and total copper of bordeaux mixture residues after weathering*

Period of exposure		Rainfall	Incubation period	Germination	Growth after transfer [1]	Appearance of residue	Amount of copper present [2]
In laboratory	In orchard						
Days	*Days*	*Inches*	*Hours*				
0	3	(3)	20	None	None	Excellent	4
0	5	1.18	24	Few buds [4]	None	do	4
0	7	1.18	24	None	Yes	do	4
0	11	1.97	20	Few buds	Yes	do	4
0	17	4.48	23	None	Yes	Very good	2
0	37	10.72	48	None	Yes	do	2
11	(3)	(3)	20	None	None	Excellent	4
35	7	.98	24	None	Yes	do	3
35	11	1.17	24	None	Yes	do	4
35	18	1.17	20	Few buds	Yes	do	3
35	21	1.41	48	do	Yes	Good	3

[1] Due to activity of conidia inhibited, and not killed, by the treatment.
[2] Total amount of copper determined by exposing the remaining residue to nitric acid fumes, neutralizing with ammonium hydroxide, and adding chromotropic solution to the remaining liquid. The figure 4 represents the deepest color obtained with this method.
[3] None.
[4] The word "bud" indicates the primordial germ tube or the beginning of germination.

It is seen that field exposure and rainfall gradually lessened the toxicity of the spray deposits, as is usual with bordeaux mixture residues. The toxicity diminished to the point where germination was checked but the conidia were not all killed, as evidenced by the fact that germination was resumed when the environment became favorable. That age alone did not lessen toxicity is proved by the tests with the slips stored in the laboratory. From these results it may be inferred that the copper compounds that imparted the original toxicity have been diluted or washed off, leaving others that could not be dissolved sufficiently to kill all the conidia. It must be emphasized that at no time during the periods of weathering were the copper residues sufficiently free of available copper to allow free germination of the conidia. There was always sufficient available copper present to cause inhibition, at least; and that a portion of the original residue remained at the end of the weathering period was plain to the eye.

It must be added that with the residues recorded as only inhibitory in table 1 many of the conidia were actually killed. Some of the conidia showed plasmolysis of the cell contents exactly as did those killed by relatively fresh residues. From this fact one would conclude that the supply of available copper had not been evenly distributed among the conidia.

From these experiments the impression was gained that some of the copper was present in such a form as not to be available to the organisms and that the conidia have not the power to dissolve copper in this form. This was particularly evident in those experiments where the residues became scanty although the total copper present, if brought into solution by conidial secretions, should have been sufficient to be toxic to all the seeded conidia.

The data presented in table 1 do not indicate the mechanism by which the injured conidia absorbed the copper. It is shown that, irrespective of the mechanism, a point is reached in the course of

prolonged weathering of the residue where, although copper is still present, its toxicity is low compared with that in an equal quantity of unweathered residue.

Only lines 1 and 7 of table 1 relate to residues not exposed to rainfall, and hence only these are comparable with the results of Barker and Gimingham (*4*) and of McCallan (*19*). Their work consistently shows that fresh residues are directly toxic to spores, but they considered that this was due to copper brought into solution by some solvent secreted by the spores themselves. The technique used by the writers could scarcely have prevented the conidia from exerting the same dissolving action, but the results of the experiments show that on the excessively weathered residues it must have been without effect.

ABSORPTION OF AVAILABLE COPPER FROM SPRAY RESIDUES BY CONIDIA OF SCLEROTINIA FRUCTICOLA

Experience in the application of the chromotropic solution to spray residues suggested its use to demonstrate the presence of copper inside conidia that had been exposed to copper ions. Trials demonstrated that the chromotropic solution diffused easily into conidia, showing its original yellow color when no copper was present and imparting a uniform purple color to the interior of those shown by collateral tests to have been affected by copper. So far efforts to demonstrate localized deposits of copper in the affected cells have failed.

The experiments were set up in exactly the same manner as described in the foregoing section, and the conidia were allowed to germinate over a 24-hour period. At the end of the incubation period a microloop of the chromotropic solution was added to the conidial suspension in contact with the bordeaux residues. The treatment caused a deep purple color to appear upon the surface of the spray particles and in the solution surrounding the particles. When conidia killed by the copper in the residues were washed free of the treated residue and residue solution, microscopic examination showed the protoplasm stained a deep purple. When residues have been reduced in toxicity from long exposure and much washing by rain the conidia take the stain in varying degrees. Those that have become plasmolyzed become deeply stained, whereas those that have not become plasmolyzed, but the germination of which has been inhibited, appear unstained. Tests performed with residues known to contain no copper did not show the color when treated with chromotropic solution, whether or not the conidia had been killed.

The development and fixation of the purple color demonstrates the actual intake of copper by living cells. It is interesting that the copper fixed within the cells appeared to have the same power to react with the chromotropic solution as that in the solution surrounding the cells. From this it would seem that the copper may be fixed rather loosely in the plasma of the cell. On the other hand, conidia which have absorbed the copper during their growth activities have been subjected to lengthy periods of washing without lessening the density of the color inside the cells. Conidia as well as mycelium

killed prior to their contact with soluble copper readily absorb this material; but the copper is removed by washing. The copper is firmly fixed, once it is absorbed by living cells.

The data presented in table 2 are from one of a series of tests made as described. Data on exposure, rainfall, and conidial germination were obtained as for table 1. At the end of the incubation period conidia were transferred from each of the treated cover slips to potato agar. Chromotropic solution was then added to each slip and observations were made of the presence of copper within the cells, in the suspension liquid, and on the particles of the spray residue.

As indicated in table 2, scattered development of very short germ tubes, or buds,[4] was noticed toward the end of the tests with bordeaux mixture, but the conidia are shown to have been killed notwithstanding. The cell contents of all gave positive reactions for copper. These residues also gave indications of considerable copper by the nitric acid test. The parallel tests of zinc-lime were included to show the absence of effect of the excess lime as mentioned on page 521. Zinc-lime is toxic to these conidia for a day or so, and it is believed that the end of this period of toxicity indicates the change of alkalinity due to the change of lime in the residue to calcium carbonate. With zinc-lime residues no indications of the presence of copper were obtained in the conidia, in the solution, or on the spray particles.

TABLE 2.—*Presence of copper inside the conidia of Sclerotinia fructicola and its effect on their subsequent growth after a 24-hour incubation period*

Residue	Days weathered	Rainfall (total)	Germination	Subsequent growth on potato agar	Fixation of copper in cell contents [1]
		Inches	*Percent*		
Zinc-lime 4–4–50	1	0.00	0	Excellent	None.
	5	.02	85	do	Do.
	8	.02	97	do	Do.
	12	.17	15	do	Do.
	15	.67	98	do	Do.
	21	1.16	96	do	Do.
Bordeaux 2–4–50	1	.00	0	None	Marked.
	5	.02	0	do	Do.
	8	.02	[2]	do	Do.
	12	.17	[2]	do	Do.
	15	.67	[2]	do	Do.
	21	1.16	[2]	do	Do.

[1] Based on depth of purple color in test spores following treatment with chromotropic solution.
[2] Few buds (primordial germ tubes).

The data in table 2 demonstrate the ability of the bordeaux residues to resist weathering and to furnish a continual supply of available copper. Not a great deal of precipitation occurred during the long period of exposure, and although at times slight evidence of germination was noted, the amount of available copper in the precipitates was not greatly diminished at any time during the test; this probably accounts for the greater toxicity of these residues as compared with those cited in table 1.

[4] The term "bud" refers to the primordial germ tube, or beginning of the germ tube.

EFFECT OF CELL ACTIVITY

In the work presented in table 1 it was noticed that after the residues had been subjected to a certain amount of weathering the conidia of a test suspension were not all affected alike. The escape of a few when nearly all were killed and the killing of a few when nearly all escaped suggested that some conidia had taken up copper more readily than others, and the color produced in them by the chromotropic solution confirmed this. To find the cause of this variation the work presented in table 3 was undertaken.

TABLE 3.—*Effect of cell activity on the absorption of copper by the conidia of Sclerotinia fructicola*

Incubation period (hours)	Germination of checks	Subsequent growth of checks	Germination of treated conidia	Treated cells absorbing copper [1]	Subsequent growth of treated conidia
	Percent		*Percent*	*Percent*	
0.0	0	Excellent			
1.0	7	do	0	13	Excellent.
2.0	25	do	0	41	Do.
3.0	41	do	0	65	Do.
4.5	74	do	0	72	None.
23.5	80	do	0	80	Do.

[1] Based upon the development of a purple color inside the test spores following treatment with chromotropic solution.

Two parallel series of germination tests were made. The cover slips in one series were without spray residue and were held as checks, whereas those in the other series were sprayed with bordeaux mixture and dried. All were started together, and at definite intervals one cover slip of each series was examined for germination. After the percentage of germinated conidia had been recorded, some of the conidia were transferred from the check and the treated cover slips to potato agar. The remainder of the conidia in contact with the spray residue were treated with chromotropic solution and examined under the microscope for evidence of copper absorption (table 3).

It was observed that conidia taken from the sprayed slips near the end of the test did not show any subsequent growth, whereas those transferred earlier did. From the data on germination of the check conidia (column 2 of table 3) it is apparent that the number of conidia that germinated in the early part of the test corresponded roughly with the number of spores that absorbed copper (column 5). It appears from this that those conidia that did not become active had not absorbed copper, since the chromotropic solution did not stain their protoplasm, but they were capable of germination and growth when transferred to a suitable medium.

To obtain further information on the relation of absorption to toxicity and to be sure that lack of absorption was not caused by a lack of soluble copper in contact with the conidia, the following experiments were conducted. Conidia from a string bean culture 8 days old were suspended in undiluted prune juice. This suspension was placed in the bottom of a beaker as a thin film so that germination or activity could proceed with an abundant supply of oxygen at room temperature.

At intervals drops of this suspension, which was of such density that about 50 conidia occupied a low-power objective field, were transferred to glass slides and an equal amount of a 2-percent copper sulphate solution was added. Thus the final concentration of copper sulphate was 1 percent. After an absorption period of 30 minutes the conidia were washed free of the copper salt and nutrient medium and then treated with the chromotropic solution. Records were then made of the conidia colored by the chromotropic solution and of germination or lack of germination, and finally some of the treated cells were transferred to potato agar. The results, as indicated in table 4, clearly show that although sufficient copper ions were present to cause toxicity to all the conidia, nevertheless in the early stages of germination many of the conidia did not absorb the copper ions. Absorption was correlated with germination activity, as is seen by comparing the percentage of conidia showing the color reaction and the percentage of germination in the check at the end of the same incubation periods. The observations clearly demonstrate that only those cells that become very active absorb the soluble copper. This was again apparent when some of the cells that had pushed out their primordial germ tubes and others that were obviously just beginning this process became deeply stained, while all the others, still dormant, remained unstained. As soon as all the conidia had become colored no subsequent growth appeared in any of the transfers to nutrient potato agar. One cannot escape the conclusion that the coloration is an indication of the absorption of a lethal dose of copper.

TABLE 4.—*Absorption of copper ions, from a 1-percent solution of copper sulphate, by the conidia of Sclerotinia fructicola*

Time of test [1]	Conidia	Check conidia germinating	Treated conidia colored [2]	Subsequent growth of treated conidia	Subsequent growth of check conidia
1934					
Sept. 28:	*Number*	*Number*	*Number*		
11 a. m.	69	0	0	Excellent	Excellent.
12 m.	76	12	18	do	Do.
1 p. m.	53	35	41	do	Do.
2 p. m.	62	62	62	None	Do.
3 p. m.	73	71	73	do	Do.
4 p. m.	70	68	70	do	Do.
Sept. 29:					
9:20 a. m.	62	0	0	Excellent	Do.
9:30 a. m.	56	0	10	do	Do.
9:50 a. m.	62	0	14	do	Do.
10 a. m.	75	6	21	do	Do.
10:20 a. m.	69	18	31	do	Do.
10:40 a. m.	71	21	36	do	Do.

[1] 2 separate tests.
[2] Colored by the reaction of copper with chromotropic solution.

It is also apparent from the combined results of these experiments that not all the conidia began germination at the same time. In residues of bordeaux mixture, where the supply of soluble copper may become temporarily depleted, those conidia that start germination first may absorb a lethal quantity of the available copper, while those that start later may find the supply of this copper lowered to a point where only inhibition results. It is even possible that some conidia may escape entirely during this temporary depletion. It appears

from this that the available copper of bordeaux mixture is not readily or quickly replaced. These factors, therefore, may be responsible for the inhibitory but nonlethal effects of potentially fungicidal copper residues so frequently encountered under field conditions. As long as a sufficient residue is present, these periods of depletion of available copper must in the main be only transitory, since it has been found that a supply of available copper will begin to accumulate as soon as those factors causing removal of the available copper have ceased to operate.

BEHAVIOR OF GERM TUBES IN CONTACT WITH BORDEAUX MIXTURE RESIDUES

The procedure just described was also applied to conidia that had been incubated in a favorable medium until germ tubes had formed. In this way it was sought to compare the susceptibility of the germ tubes to the action of the spray residues with that of the conidia before and during germination, and again to demonstrate the intake of copper by means of the chromotropic solution.

In this part of the study two types of experiments were conducted. In both sets the procedure was essentially the same, in that the conidia were germinated in dilute prune juice before their transfer to the bordeaux mixture residues.

The residues used in both of these experiments were not weathered in the orchard environment, but had been prepared and stored in the laboratory fully a month before their use. The excess lime at that time had become fully carbonated, as was demonstrated by tests with phenolphthalein. The residues used carried a good supply of available copper, as indicated by the reaction with chromotropic solution.

In the first set of experiments the conidia were transferred directly without freeing them of any adhering metabolic and nutrient products, while in the second set the germinated conidia with their germ tubes were washed for 24 hours in tap water to free them from these materials insofar as practicable before their transfer to the bordeaux mixture residues.

In the first set of experiments, representative germ tubes were measured at the time of the transfers and again after certain intervals, depending upon the experiment. Some of the treated germinated conidia were tested with chromotropic solution and some were transferred to a suitable culture medium. In this manner observations were conducted on absorption of copper by the germ tubes and its effect on their subsequent growth. The results of such an experiment are presented in table 5.

The measurements recorded show that while the transfer had little effect upon the growth of the control germ tubes, the growth of the others was stopped almost instantly when they came in contact with the bordeaux mixture residues. Their failure to grow when transferred to a suitable medium indicated that the copper absorbed was lethal.

The conidia and germ tubes that had been in contact with the residues showed a slight plasmolysis of the protoplasm and became colored a deep purple upon treatment with chromotropic solution. The distribution of the copper, as indicated by the purple color, was found to be uniform throughout the entire protoplasmic system. The cell walls appeared not to absorb or fix the copper.

TABLE 5.—*Effect of absorption of copper by the germ tubes of conidia of Sclerotinia fructicola on the length growth of the tubes and on subsequent activity*

Time of measurement [1]	Check germ tubes		Treated germ tubes		
	Length growth	Subsequent growth	Length growth	Absorption of copper [2]	Subsequent growth [3]
	μ		μ		
11 a. m.	129	Excellent.	108	Some faintly colored	Excellent.
1 p. m.	138	...do...	93	Some well colored but most faintly colored.	None.
2 p. m.	201	...do...	86	Most well colored; some faintly.	Do.
3:30 p. m.	194	...do...	108	Most well colored	Do.
10:30 a. m. (following day)	720	...do...	108	All well colored	Do.

[1] Germinated conidia were transferred at 10 a. m.
[2] Based upon the depth of the purple color following treatment with chromotropic solution.
[3] Subsequent growth determined by transferring some of the check and some of the treated conidia, with their germ tubes, to potato hard agar slants.

In the second set of experiments the germinated conidia, with their attached germ tubes, as already stated, were washed for 24 hours before being transferred to the clean or to the residue-covered slips. This procedure was designed to prevent, as far as possible, the staling products and nutritional materials from either increasing or lessening the quantity of available copper in the bordeaux-mixture residues. With such a treatment a small quantity of carbon dioxide might accumulate in the vicinity of each conidium, but it appears hardly likely that this amount could have any great influence.

The germinated conidia were transferred from the washed suspension to the clean or to the residue-covered slips. After intervals of ½ hour, 1 hour, 4 hours, and 24 hours, some of the check and some of the treated organisms were transferred from the slips to potato hard agar to determine the toxic effects of the copper. At the same time the remainder were treated with chromotropic solution and examined for copper within the cells.

The results showed that the available copper was absorbed by the germ tubes and conidia in lethal quantities during every period of contact with the residue. Thus the effect was even more pronounced than when the staling and nutritional products were not removed. The excellent growth of all the untreated transfers showed that the washing treatment was in no way deleterious.

In the section just preceding it was shown that the absorption of copper by conidia took place at so early a stage of activity that no evidence of initial germination could be seen under the microscope, either in conidia that had already absorbed an injurious quantity of copper or in the controls that had been incubated for the same length of time. In the work just described it is shown that when conidia that are already germinating are placed in contact with a source of available copper they too are immediately killed, as is evidenced by the instant cessation of tube growth.

DISCUSSION

From the work reported in this paper it is evident that the residue is altered by exposure to weathering, since it then behaves somewhat differently from freshly formed residue. The work presented here

aslo shows that when this change has gone so far that sufficient quantities of available copper are not present the application of additional sprays to maintain continuous protection is required. The changed behavior indicates that some form of copper present in the beginning is not present at the end of prolonged weathering and that the forms of copper that remain are not able to maintain the original high concentration in the solution in contact with them.

The proportion of highly soluble copper in the fresh deposit and the rapidity with which it can be exhausted by washing remain to be determined. From this work it appears that the amount is not large and that the appearance of the residue does not give warning of its depletion. As weathering progresses, an increasing proportion of the conidia seeded upon the residue retain their viability, although germination is prevented.

It must be emphasized, therefore, that the bordeau mixture residues which are commonly applied to plants and which have been found to be injurious to many of them, according to the environmental conditions encountered, are toxic to many of the fungus spores which come in contact with them from the moment they are applied. These experiments have clearly shown the reasons for this, and it appears certain that the vagrant fungus conidia that fall on these residues come in contact with sufficient available copper to cause toxicity, provided the residues are not unduly washed or depleted.

Other evidence in regard to the availability or nonavailability of the copper of spray residues is found in the results of tests with the chromotropic solution. The direct application of this reagent gives a color the depth of which is roughly proportional to the toxicity as determined in the usual way.

The adherence of most of the residue is shown to be quite satisfactory. Although after 5 days the seeded spores showed themselves able to germinate after transfer (table 1), the residues continued to prevent germination during 37 days in spite of the number of heavy rains. In table 2, on the other hand, no growth was recorded after transfer on bordeaux mixture residues weathered for 21 days. This is attributed to the fact that decidedly less rain fell during the course of this experiment, and very little during the first 12 days (table 2). It also suggests that the foliage injury which often follows long periods of fog, heavy dew, or showers may be caused by the maintenance of a copper solution upon the leaves.

It has been observed repeatedly that the absorption of copper takes place only in cells that become active and not in those that are dormant or inactive. This has been very well shown in the experiments. The results obtained with cells washed relatively free of their metabolic products clearly demonstrate that such cells are capable of absorbing directly from bordeaux mixture residues a form of copper causing a lethal effect. These copper materials, when in rather low concentrations, were found to be only inhibitory in effect.

The mechanism of the lethal effect has not been clearly worked out. It is certain that the copper becomes dispersed throughout the protoplasmic mass, for the color produced when the loosely combined soluble copper reacts with the chromotropic molecule is evenly distributed. These experiments show that inactivation follows the absorption of the copper and that the activity of the cell menstruum is responsible for the intake of the copper ions. It has also been

observed that the reaction of the copper ions with the cell menstruum is a permanent one, for after the death of the cells no amount of washing frees the injured cells of the copper that has been absorbed. Nevertheless the copper is not so firmly bound in its combination with the elements of the cytoplasm as to prevent its usual response to treatment with the chromotropic solution.

It has been the constant experience of the writers that at times the amount of copper in solution is only of such magnitude as to cause inhibition. The study of inhibition is an interesting one, since it must take into consideration an entirely different set of conditions from those that obtain with the active cell. It is difficult to understand the phenomenon of inhibition when the theory of toxicity is applied to it, except as something different in kind as well as in degree from toxicity.

While these studies offer a satisfactory explanation of the effect of the available copper of bordeaux mixture residues on sensitive organisms, they do not attempt to define those systems concerned with organisms apparently resistant to copper. Such systems require special study.

SUMMARY AND CONCLUSIONS

A study is presented in this paper of the effect on the conidia of *Sclerotinia fructicola* (Wint.) Rehm of the available copper inherent in the residues of bordeaux mixture that have been exposed to weathering and aging.

This study also includes the effect on germinated conidia of *Sclerotinia fructicola* of the available copper inherent in the residues of bordeaux mixture that have been aged in the laboratory.

A new procedure, namely, exposure of the residues to the environment of the host plants, has been added to the usual methods of estimating the effect of fungicides. This method furnishes a means of weathering residues of fungicides under the same environmental conditions as those which affect the spray residues on the host plants.

As a further precaution in the proper evaluation of the effects of fungicides on fungus spores, some of the test spores were transferred to a suitable nutrient medium in order to determine whether the effect of the test materials was toxic or merely inhibitory.

It has been determined, confirming the work of others, that the soluble copper inherent in the precipitates of the residues of bordeaux mixture is in the proportion of at least four parts per million by volume of the original mixture.

The soluble copper in residues, in solution, and in the protoplasm of affected conidia has been detected by the use of a very sensitive reagent, namely, the sodium salt of nitroso-chromotropic acid, which is known as the "chromotropic solution."

It has been demonstrated that the copper of the residues of bordeaux mixture is absorbed by the conidia of *Sclerotinia fructicola*, causing death or inhibition according to the amount of copper available.

The absorption of the copper by the conidia from the residues of bordeaux mixture is correlated with the initiation of activity, and cells that remain dormant or are delayed in their activity do not absorb copper.

Conidia of *Sclerotinia fructicola* have been found to be inhibited in their growth when the supply of copper has not been quite sufficient

to cause toxicity. These inhibited conidia readily become active when transferred to a suitable culture medium.

It is indicated that where the quantity of soluble copper in a depleted residue is small, it may be exhausted by absorption into the conidia that begin their activity first, and that conidia that become active later escape the injurious effect.

When actively growing conidia were transferred to an aged bordeaux mixture residue, but one in which the inherent soluble copper had not been depleted, both the conidial and attached germ-tube cells absorbed the copper with lethal effects.

The absorption of the lethal dose of soluble copper from aged residues by the germ tubes of the conidia of *Sclerotinia fructicola* was found to cause an immediate cessation of germ-tube elongation.

The soluble copper that is absorbed by the conidia and germ tubes has been found to be distributed evenly throughout the protoplasmic mass, which stains purple when treated with chromotropic solution.

The color that is formed by the combination of chromotropic solution with the copper absorbed in the cells has been found to be similar to that which simple copper ions form with chromotropic solution in vitro.

It is concluded that in freshly deposited bordeaux mixture residues there is present a component bearing available copper which is exhausted by washing with rains but not by aging. After it is lost, conidia that come to rest in contact with the residue are inhibited in germination but are not killed, and can germinate freely when removed from this contact. While it is still present, conidia take up copper before any visible evidence of vital activity is observed. From this it appears that the conidial cells are not capable of secreting a substance which will dissolve the inert copper of these washed residues.

LITERATURE CITED

(1) Allison, F. E., Hoover, S. R., and Burk, D.
1933. A respiration coenzyme. Science (n. s.) 78: 217–218.

(2) Ansbacher, S., Remington, R. E., and Culp, F. B.
1931. Copper determination in organic matter. Indus. and Engin. Chem., Analyt. Ed. 3: 314–317.

(3) Bain, S. M.
1902. The action of copper on leaves with special reference to the injurious effects of fungicides on peach foliage: a physiological investigation. Tenn. Agr. Expt. Sta. Bull. 15: [21]–106, illus.

(4) Barker, B. T. P., and Gimingham, C. T.
1911. The fungicidal action of bordeaux mixture. Jour. Agr. Sci. [England] 4: [76]–94.

(5) Branas, J., and Dulac, J.
1934. Sur le mode d'action des bouillies cupriques. Rôle de la desiccation. Prog. Agr. et Vitic. 100: 642–644.

(6) Brenner, C.
1920. Beitrag zur theorie der farblacke. Helvetica Chim. Acta 3: 90–103.

(7) Butler, O.
1914. Bordeaux mixture: I. physico-chemical studies. Phytopathology 4: [125]–180, illus.

(8) Callan, T., and Henderson, J. A. R.
1929. A new reagent for the colorimetric determination of minute amounts of copper. Analyst 54: 650–653.

(9) Cherbuliez, E., and Ansbacher, S.
1930. Sur le dosage du cuivre dans des matières organiques. Helvetica Chim. Acta 13: 187–194.

(10) CLARK, J. F.
1902. ON THE TOXIC PROPERTIES OF SOME COPPER COMPOUNDS WITH SPECIAL REFERENCE TO BORDEAUX MIXTURE. Bot. Gaz. 33: 26–48, illus.

(11) CRANDALL, C. S.
1909. BORDEAUX MIXTURE. Ill. Agr. Expt. Sta. Bull. 135, pp. 201–296, illus.

(12) ELVEHJEM, C. A., and LINDOW, C. W.
1929. THE DETERMINATION OF COPPER IN BIOLOGICAL MATERIALS. Jour. Biol. Chem. 81: 435–443.

(13) FEIGL, F.
1931. QUALITATIVE ANALYSE MIT HILFE VON TÜPFELREAKTIONEM; THEORETISCHE GRUNDLAGEN UND PRAKTISCHE AUSFÜHRUNG. 387 pp., illus. Leipzig.

(14) HOCKENYOS, G. L.
1931. SOLUBILITY OF BORDEAUX. Phytopathology 21: 231–234.

(15) ——— and IRWIN, G. R.
1932. STUDIES ON BORDEAUX DEPOSITION. Phytopathology 22: 857–860.

(16) HOLLAND E. B., DUNBAR, C. O., and GILLIGAN, G. M.
1929. SUPPLEMENTS FOR COPPER FUNGICIDES. Mass. Agr. Expt. Sta. Bull. 252, pp. [94]–112.

(17) LUTMAN, B. F.
1912. THE COVERING POWER OF THE PRECIPITATION MEMBRANES OF BORDEAUX MIXTURE. Phytopathology 2: [32]–41, illus.

(18) MCCALLAN, S. E. A.
1930. STUDIES ON FUNGICIDES. II. TESTING PROTECTIVE FUNGICIDES IN THE LABORATORY. N. Y. (Cornell) Agr. Expt. Sta. Mem. 128: 8–24, illus.

(19) ———
1930. STUDIES ON FUNGICIDES. III. THE SOLVENT ACTION OF SPORE EXCRETIONS AND OTHER AGENCIES ON PROTECTIVE COPPER FUNGICIDES. N. Y. (Cornell) Expt. Sta. Mem. 128: 25–79, illus.

(20) ——— and WILCOXON, F.
1932. THE PRECISION OF SPORE GERMINATION TESTS. Boyce Thompson Inst. Contrib. 4: 233–243, illus.

(21) MARTIN, H.
1928. THE SCIENTIFIC PRINCIPLES OF PLANT PROTECTION. 316 pp. London.

(22) ———
1932. STUDIES UPON THE COPPER FUNGICIDES. I. THE INTERACTION OF COPPER SULPHATE WITH CALCIUM HYDROXIDE. Ann. Appl. Biol. 19: 98–120, illus.

(23) MILLARDET, [A.], and GAYON, [U.]
1885. TRAITEMENT DU MILDIOUS PAR LE MÉLANGE DE SULFATE DE CUIVRE ET DE CHAUX. Jour. Agr. Prat. 49 (t. 2): 707–710.

(24) PICKERING, S. U.
1907. THE CHEMISTRY OF BORDEAUX MIXTURE. Jour. Chem. Soc. [London] Trans. 91: 1988–2001.

(25) POSNJAK, E., and TUNELL, G.
1929. THE SYSTEM, CUPRIC OXIDE-SULPHUR TRIOXIDE-WATER. Amer. Jour. Sci. (5) 18: 1–34, illus.

(26) PROUST, [J. L.]
1800. RECHERCHES SUR LE CUIVRE. Ann. Chim. et Phys. 32: 26–54.

(27) RIBÉREAU-GAYON, J.
1933. SUR LA SOLUBILITÉ DES COMPOSÉS CUIVRIQUES DES BOUILLIES ANTI-CRYPTOGAMIQUES. Compt. Rend. Acad. Sci. [Paris] 197: 267–268.

(28) SCHMIDT, E. W.
1924. WIE KOMMT DIE WIRKUNG DER KUPFERKALKBRÜHE ZUSTANDE? Centbl. Bakt. [etc.] (II) 61: 356–367.

(29) WHETZEL, H. H., and MCCALLAN, S. E. A.
1930. STUDIES ON FUNGICIDES. I. CONCEPTS AND TERMINOLOGY. N. Y. (Cornell) Agr. Expt. Sta. Mem. 128: 3–7.

INHERITANCE OF CHLAMYDOSPORE CHARACTERISTICS IN OAT SMUT FUNGI [1]

By C. S. HOLTON [2]

Agent, Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

The nature of inheritance in the smut fungi has been the subject of rather extensive investigations in recent years. Primary emphasis in these investigations has been placed on a study of the segregation of factors for sex and cultural characters, whereas little has been done towards determining the nature of inheritance of chlamydospore characteristics. In previous reports [3] the writer has presented preliminary evidence to show that certain chlamydospore characteristics in the oat smut fungi are inherited according to the Mendelian law of segregation and recombination. Results are now available from more extensive investigations and are here reported.

MATERIAL AND METHODS

Loose smut of oats, *Ustilago avenae* (Pers.) Jens., covered smut of oats, *U. levis* (Kell. and Sw.) Magn., and the "buff smut" fungus [4] were used in these studies. They hybridize readily, and their respective chlamydospore characteristics are morphologically distinct. *U. avenae* has echinulate brown chlamydospores; *U. levis* has smooth brown chlamydospores; and the buff smut fungus has smooth hyaline chlamydospores. In hybrids between these species, therefore, it is possible to study the nature of segregation and recombination of factors for chlamydospore markings and color both in single and in double factor pairs.

Hybrid material was obtained by inoculating 25 oat seedlings (*Avena sativa* L.) with crosses between monosporidial lines of *Ustilago levis* and the buff smut fungus, between *U. levis* and *U. avenae*, and between *U. avenae* and the buff smut fungus. The ratios in the F_2 segregations were determined on the basis of types of individuals (chlamydospores) produced by inoculating with F_1 chlamydospores or with crosses between F_1 monosporidial lines. The observed distribution of the F_2 populations of all hybrids was compared with the expected distribution and tested by Fisher's formula for testing goodness of fit, $X^2 = S\left(\frac{x^2}{m}\right)$. [5]

[1] Received for publication Oct. 7, 1935; issued April 1936. Cooperative investigations by the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Washington Agricultural Experiment Station.

[2] The writer is indebted to Dr. E. F. Gaines, cerealist, and A. M. Schlehuber, of the Washington Agricultural Experiment Station, for assistance in analyzing the data, and to Dr. F. D. Heald, head of the Division of Plant Pathology, and Dr. H. F. Clements, associate professor of botany, Washington Agricultural Experiment Station, for critical reading of the manuscript.

[3] HOLTON, C. S. STUDIES IN THE GENETICS AND THE CYTOLOGY OF USTILAGO AVENAE AND USTILAGO LEVIS. Minn. Agr. Expt. Sta. Tech. Bull. 87, 34 pp., illus. 1932.

——— INHERITANCE OF CHLAMYDOSPORE CHARACTERISTICS IN OAT-SMUT FUNGI. (Abstract) Phytopathology 23: 16. 1933.

[4] HOLTON, C. S. HYBRIDIZATION AND SEGREGATION IN THE OAT SMUTS. Phytopathology 21: 835-842, illus. 1931.

[5] FISHER, R. A. STATISTICAL METHODS FOR RESEARCH WORKERS. Ed. 2, rev. and enl., 269 pp., illus. Edinburgh and London. 1928. See p. 75.

EXPERIMENTAL DATA

USTILAGO LEVIS × BUFF SMUT

In hybrids between *Ustilago levis* (chlamydospores brown) and the buff smut fungus (chlamydospores hyaline) the F_1 chlamydospores were brown, indicating dominance of the factor for brown. In these hybrids approximately 50 percent of the F_1 sporidia were viable. Two or more monosporidial lines were obtained from each of 8 F_1 chlamydospores, giving a total of 22, and these F_1 monosporidial lines were crossed in all sexually compatible combinations to obtain the F_2 generation.

As indicated in table 1, the F_2 population was distributed into two classes. In four of the chlamydospores (45, 47, 49, and 57) segregation for sex occurred in the first division while in the other four (50, 53, 54, and 55) segregation for sex occurred in the second division of the diploid nucleus. In all the F_1 chlamydospores, except 49 and 54, segregation for color occurred in the second division of the diploid nucleus. In chlamydospores 45, 47, 54, and 57 segregation for sex and color occurred in separate nuclear divisions, while in chlamydospores 50, 53, and 55 segregation for sex and color occurred in the same (second) division of the diploid nucleus. It is therefore evident (table 1) that the F_2 population of these seven chlamydospores was distributed into two classes in a 3:1 ratio. In chlamydospore 49, however, segregation for sex and color occurred in the first division of the diploid nucleus, and consequently the F_2 population of this spore was brown. Nevertheless, segregation for color and sex occurred in the F_2 of this spore in such a way as to produce brown and hyaline spores in the F_3. On the basis of these data it is apparent that the F_2 distribution of color in *Ustilago levis* × buff smut hybrids is in a simple 3:1 ratio.

TABLE 1.—*Segregation of factors for color* [1] *and sex in eight chlamydospores of a Ustilago levis × buff smut hybrid*

F_1				F_2		F_1				F_2	
Chlamydospore no.[2] and genotype	Sporidium no.	Sex [3]	Gametes	Genotype	Ratio	Chlamydospore no.[2] and genotype	Sporidium no.	Sex [3]	Gametes	Genotype	Ratio
5 (*Bb*)	1	+	*b*	*BB*	1:2:1	50 (*Bb*)	1	+	*B*	*BB*	1:2:1
	2	+	*B*	*Bb*			2	−	*b*	*Bb*	
	3	−	*b*	*Bb*			3	+	*b*	*Bb*	
	4	−	*B*	*bb*			4	−	*B*	*bb*	
47 (*Bb*)	1	+	*b*	*BB*	1:2:1	53 (*Bb*)	1	+	*b*	*BB*	1:2:1
	2	+	*B*	*Bb*			2	−	*B*	*Bb*	
	3	−	*B*	*Bb*			3	−	*b*	*Bb*	
	4	−	*b*	*bb*			4	+	*B*	*bb*	
49 (*Bb*)	1	+	*b*	*Bb*	4:0	54 (*Bb*)	1	+	*B*	*BB*	1:2:1
	2	+	*b*	*Bb*			2	−	*B*	*Bb*	
	3	−	*B*	*Bb*			3	+	*b*	*Bb*	
	4	−	*B*	*Bb*			4	−	*b*	*bb*	
57 (*Bb*)	1	+	*b*	*BB*	1:2:1	55 (*Bb*)	1	+	*b*	*BB*	1:2:1
	2	+	*B*	*Bb*			2	−	*B*	*Bb*	
	3	−	*b*	*Bb*			3	−	*b*	*Bb*	
	4	−	*B*	*bb*			4	+	*B*	*bb*	

[1] The factor for brown, carried by *U. levis*, is represented by *B*, and the factor for hyalineness, carried by the buff smut, is represented by *b*.

[2] Numbers assigned to the chlamydospores from which single sporidia were isolated.

[3] Plus (+) and minus (−) signs indicate sporidia of opposite sex.

The advantage of using crosses between F_1 monosporidial lines for determining the class distribution of the F_2 population is obvious. This advantage is lost, however, unless two or more F_1 sporidia can be obtained from each promycelium. This was possible only in the *Ustilago levis* × buff smut hybrids. It therefore became necessary to use F_1 chlamydospores of other hybrids for inoculum in order to obtain an F_2 population. To test the dependability of this method of determining the F_2 distribution, an F_2 population of the *U. levis* × buff smut hybrid, the F_2 distribution of which had previously been determined by inoculating with crosses between F_1 monosporidial lines, was obtained by inoculating with F_1 chlamydospores. By using each smutted panicle as an individual [6] the class distribution was determined. The F_2 population of 1,078 individuals (smutted panicles) was distributed into two classes, as shown in table 2.

TABLE 2.—*Segregation for color in the F_2 of the hybrid Ustilago levis × buff smut*

Color	F_2 individuals	
	Observed	Expected
	Number	*Number*
Brown (chlamydospores brown)	826	809
Buff (chlamydospores hyaline)	252	269
Total	1,078	1,078

These data are in close agreement with the expected 3:1 ratio (P=0.24) and serve to indicate the dependability of this method for determining the nature of segregation and recombination of factors for chlamydospore characteristics in oat smut hybrids.

USTILAGO AVENAE × U. LEVIS

In hybrids between *Ustilago avenae* (chlamydospores echinulate) and *U. levis* (chlamydospores smooth) the F_1 chlamydospores were echinulate, indicating dominance of the factor for echinulate spore walls over the factor for smooth spore walls. The F_2 population was obtained by inoculating with F_1 chlamydospores, and the 235 individuals (smutted panicles of the F_2) were distributed into two classes, as shown in table 3.

TABLE 3.—*Segregation for smoothness in the F_2 of the hybrid Ustilago avenae × U. levis*

Character	F_2 individuals	
	Observed	Expected
	Number	*Number*
Echinulate	185	176
Smooth	50	59
Total	235	235

[6] The panicle rather than the plant was used as the unit because both types were sometimes found on the same plant but in separate panicles.

These figures are adaptable to a theoretical ratio for the F_2 of a cross involving a single-factor difference ($P=0.18$). Apparently, then, the distribution of the F_2 population into echinulate and smooth chlamydospore classes in *U. avenae* × *U. levis* hybrids is on a simple 3:1 basis.

USTILAGO AVENAE × BUFF SMUT

In hybrids between *Ustilago avenae* and the buff smut fungus two character pairs are involved, viz, chlamydospore markings and color. Taken separately, as in *U. avenae* × *U. levis* and *U. levis* × buff smut hybrids, respectively, these character pairs give a 3:1 ratio. Therefore, in *U. avenae* × buff smut hybrids it might be expected that the typical dihybrid ratio 9:3:3:1 would be obtained. This, however, is not the case. The F_2 population, which was obtained by inoculating with F_1 chlamydospores, was distributed into three of the four expected classes, as shown in table 4.

TABLE 4.—*Segregation for color and smoothness in the F_2 of the hybrid Ustilago avenae × buff smut*

Characters	F_2 individuals	
	Observed	Expected [1]
	Number	*Number*
Brown echinulate	499	490
Brown smooth	175	164
Hyaline echinulate	0	164
Hyaline smooth	199	55
Total brown	674	654
Total hyaline	199	219
Total echinulate	499	654
Total smooth	374	219
Total individuals	873	873

[1] On the basis of the 3 classes into which the F_2 population was actually distributed, the expected numbers are as follows: Brown echinulate, 490; brown smooth, 164; hyaline smooth, 219.

If the double recessive class, hyaline smooth, had appeared in the expected proportion it might have been logical to attribute the absence of the hyaline echinulate class to lethal factors. However, there were almost four times as many smooth hyaline individuals as were expected, and the total number of hyaline individuals, as a class, was about equal to the number that was expected, on a 9:3:3:1 basis. In other words, considering the single-factor pair for color independently, the 873 F_2 individuals were distributed into two classes, brown and hyaline (table 4). The observed distribution is within the theoretical limits of a 3 : 1 ratio ($P=0.16$). The fact that all of the hyaline individuals had smooth walls seems to indicate that the expression of echinulation in the hyaline chlamydospores is suppressed. Furthermore, considering the single-factor pair for chlamydospore markings, the 873 F_2 individuals were distributed into two classes, echinulate and smooth (table 4).

Obviously, this observed distribution does not agree with the expected distribution, on a 3:1 basis, because of the absence of the hyaline echinulate class ($P<0.01$). However, on the assumption that expres-

sion of echinulation is suppressed in hyaline spores, the observed distribution of echinulate and smooth chlamydospores shown in table 4 is about what would have been expected.

If, on the other hand, hyaline echinulate spores had been produced in the proper proportion for a 9:3:3:1 ratio, then the F_2 population would have been distributed into two classes, as shown in table 5.

TABLE 5.—*Hypothetical distribution of F_2 population of hybrid Ustilago avenae × buff smut*

Character	F_2 individuals	
	Assumed as observed	Expected
	Number	*Number*
Echinulate	663	654
Smooth	210	219
Total	873	873

It is evident that the distribution assumed as observed of the F_2 population into echinulate and smooth classes, based on the assumption that hyaline echinulate individuals did occur in the expected proportion for a 9:3:3:1 ratio, is agreeable with the expected distribution for a 3:1 ratio (P=0.48). Apparently, then, there is reason to assume the presence of an inhibitor which prevents the expression of the factor for echinulation in the hyaline spores of the buff smut fungus. If this is true, it would be expected that the F_2 population of *Ustilago avenae* × buff smut hybrids would be distributed in proper proportions for a 9:3:4 ratio. The 873 individuals of this cross were distributed into three classes, brown echinulate, brown smooth, and hyaline smooth (table 4).

That the distribution of the F_2 population of the *Ustilago avenae* × buff smut hybrid is in agreement with the expected distribution for a 9:3:4 ratio (P=0.26) seems to justify the assumption that an inhibitor is present which suppresses echinulation in the hyaline chlamydospores. If the inhibitor hypothesis is correct, then half of the hyaline gametes would carry the factor for echinulation and should produce echinulate brown F_1 chlamydospores when crossed with *U. levis*. Crosses of this type, which would be final proof for the inhibitor hypothesis, have not as yet been made.

SUMMARY

The factor for brown is dominant over the factor for hyaline chlamydospores in hybrids between covered smut of oats, *Ustilago levis* (chlamydospores brown, smooth), and the buff smut fungus (chlamydospores hyaline, smooth), and the distribution of the F_2 population is on a simple 3:1 basis.

The factor for echinulate spore walls is dominant over the factor for smooth spore walls in hybrids between loose smut of oats, *Ustilago avenae* (chlamydospores echinulate), and covered smut of oats, *U. levis* (chlamydospores smooth), and the F_2 population of echinulate and smooth chlamydospores was distributed on a 3:1 basis.

In hybrids between *Ustilago avenae* (chlamydospores brown, echinulate) and the buff smut fungus (chlamydospores hyaline, smooth) factors for echinulation and brown are dominant over factors for smoothness and hyalineness, respectively. The F_2 population was distributed into echinulate brown, smooth brown, and smooth hyaline classes in a 9:3:4 ratio. The evidence seems to indicate that the presence of an inhibitor prevents the expression of echinulation in the hyaline chlamydospores of the buff smut fungus.

PENETRATION OF TRICHODERMA LIGNORUM INTO SAPWOOD OF PINUS TAEDA [1]

By MAE SPRADLING [2]

Assistant scientific aide, Division of Forest Pathology, Bureau of Plant Industry United States Department of Agriculture

INTRODUCTION

Trichoderma lignorum (Tode) Harz is the most common mold found growing on the unseasoned sapwood of pine. During warm, humid weather and under improper methods of seasoning, the sapwood surfaces of some species may be partially covered by this fungus within a few days after sawing. In general, lumbermen disregard the presence of this fungus because they consider it a superficial growth causing only a surface discoloration which disappears after the lumber is seasoned. So far as the writer knows no experimental data have been published which show that *Trichoderma lignorum* penetrates wood. Gerry,[3] however, showed that five other wood-inhabiting molds penetrated both hardwoods and softwoods. Falck[4] stated that water-stored wood is often attacked to a certain degree by the so-called green "Holzschimmel" (*Trichoderma lignorum*). He also stated that this mold may spread very rapidly over the outer surface and to the interior of the wet wood without influencing the strength of the wood.

Trichoderma has frequently been isolated by the writer from the interior of wood, indicating that it is more than a superficial growth on the wood. The following experiments were therefore undertaken to determine the extent and rapidity at which *Trichoderma lignorum* would grow through the sapwood of loblolly pine (*Pinus taeda* L.). The first results obtained were sufficient to prove that the fungus penetrates steam-sterilized wood rapidly. Subsequent investigations were made to determine the effect of steam sterilization on the susceptibility of the wood to the invasion of the fungus, and the part played by the moisture content of the wood.

EXPERIMENTAL DATA

METHODS

Five strains of *Trichoderma lignorum* were isolated by the spore-dilution method from southern yellow pine chips collected in mill yards in Mississippi and Louisiana. The cellulose-dissolving ability of these strains was tested on McBeth and Scales' cellulose agar.[5] The strain that cleared the agar most rapidly (no. 5) and the strain that

[1] Received for publication Dec. 2, 1935; issued April 1936. In cooperation with the Forest Products Laboratory, Forest Service, U. S. Department of Agriculture.

[2] The writer wishes to express her appreciation to C. A. Richards, Carl Hartley, R. M. Lindgren, and other members of the Division of Forest Pathology for helpful suggestions received during the investigation reported in this paper.

[3] GERRY, E. FIVE MOLDS AND THEIR PENETRATION INTO WOOD Jour. Agr. Research 26: 219-229, illus. 1923.

[4] FALCK, R. UEBER DEN EINFLUSS DES FLÖSSENS AUF DIE WIDERSTANDSFÄHIGKEIT DES RAUHOLZES GEGEN TROCKENFÄULE UND ÜBER DEN HOLZSCHUTZ DURCH SCHIMMELBEFALL UND DIFFUSIONSTRÄNKUNG. Mitt. Forst. u. Forstwiss. 11: 480-485, illus. 1931.

[5] MCBETH, I. G., and SCALES, F. M. THE DESTRUCTION OF CELLULOSE BY BACTERIA AND FILAMENTOUS FUNGI. U. S. Dept. Agr., Bur. Plant Indus. Bull. 266: 27-28. 1913.

did not clear it within 10 days after inoculation (no. 3) were selected for use in the penetration studies.

Experimental blocks, 2 by 2 by 2 inches, were quarter-sawed from the sapwood of the *Pinus taeda* logs collected in Louisiana and Florida. The wood was free from infection, the rate of growth was medium (from 4½ to 6 annual rings per inch), and the grain was fairly straight. The ends of the freshly cut logs had been treated with ethyl mercuric chloride and painted with filled hardened gloss oil to prevent invasion of fungi and loss of moisture.

Blocks in one group were selected at random and steamed for 30 minutes at 100° C. (atmospheric pressure). Blocks in another group were selected so that those steamed for 30 minutes at 121° (15 pounds pressure) could be compared with longitudinally and tangentially matched unsteamed blocks. In a third group, blocks were steamed 30 minutes at 100° and compared with others that had been steamed 30 minutes at 121° and also with unsteamed blocks. Figure 1 shows how the blocks were selected.

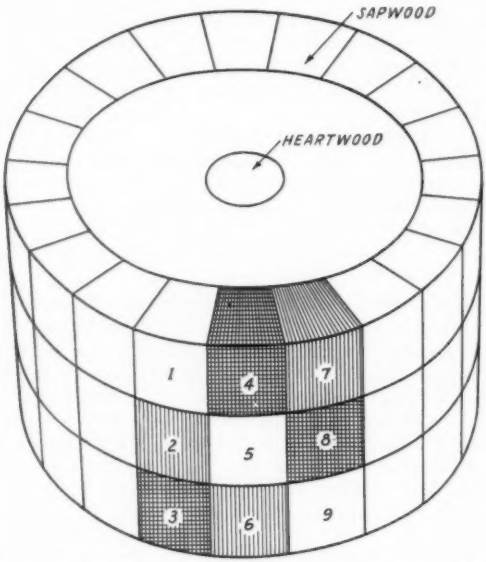

FIGURE 1.—Diagram showing how 2-inch cubes were cut from the log. Not shaded (1, 5, 9), not steamed; lightly shaded (2, 6, 7), steamed 30 minutes at 100° C.; heavily shaded (3, 4, 8), steamed 30 minutes at 121°.

To prevent *Trichoderma* from entering any side of the block except the one upon which the inoculation was to be made, all sides except that one were dipped in a hot bath of paraffin (about 100° C.) and held for 15 seconds, then immediately transferred to a cooler paraffin bath (about 60°) for 30 seconds. The object of the first paraffin bath was to penetrate the surface of the block sufficiently to hold the thicker paraffin film formed in the second bath.

The blocks were placed in sterile glass jars, 5 by 5 by 10 inches, containing 35 cc of distilled water. They were prevented from coming into direct contact with the water by means of glass supports. A layer of cotton was placed between the glass stopper and the rim of the jar.

As soon as the steamed blocks were cool they were inoculated with approximately 0.2 cc of a heavy spore suspension of *Trichoderma lignorum* placed on the center of the unparaffined side of each block. They were then incubated at from 25° to 30° C. for different periods.

At the end of the incubation period small cubes which were to be sectioned for microscopical examination were sawed from the inocu-

lated blocks (fig. 2). These cubes were boiled 15 minutes in distilled water and preserved in a 50–50 glycerin-alcohol mixture. From 10 to 20 radial sections 30μ thick were then taken from the cubes and examined under the microscope for the presence and depth of penetration of *Trichoderma* mycelium. The sections were stained 15 minutes in Pianeze III b,[6] dehydrated with alcohol, cleared with a solution of carbol-turpentine, treated with xylol, and mounted in Canada balsam.

Moisture determinations [7] of the wood were made at the end of the incubation period. The moisture samples consisted of a tangential and a radial side which remained after the center was removed from the 2-inch cube (fig. 2).

RESULTS

The rate and extent of penetration of *Trichoderma lignorum*, strains nos. 3 and 5, into *Pinus taeda* sapwood that had been steamed 30 minutes at 100° C. are recorded in table 1. At the end of 72 hours both strains of the fungus had penetrated a 2-inch block which had been inoculated on the tangential side. At the end of 60 hours, strain no. 5 had penetrated a block inoculated on the transverse side, while strain no. 3 had penetrated only three-fourths of that distance.

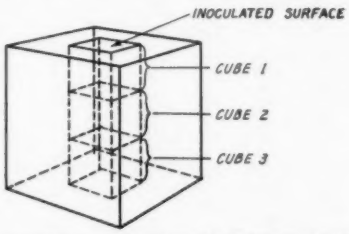

FIGURE 2.—Method of cutting blocks inoculated with *Trichoderma* in preparation for sectioning.

The mycelium was present in the ray parenchyma cells, ray tracheids, wood tracheids, and resin ducts. The hyphae were most abundant in the ray parenchyma and wood tracheids (fig. 3). This was true for both strains. No evidence of direct penetration was seen. The mycelium appeared to pass through the natural openings.

TABLE 1.—*Rate of penetration of Trichoderma lignorum into Pinus taeda sapwood steamed 30 minutes at 100° C.*

Incubation period (hours)	Strain no.	Distance penetrated		Incubation period (hours)	Strain no.	Distance penetrated	
		Radially	Longitudinally			Radially	Longitudinally
		Inches	*Inches*			*Inches*	*Inches*
24	3	[1]	0.00	60	3	0.88	1.50
	5	[1]	.00		5	.88	2.00
48	3	0.19	1.06	72	3	2.00	----------
	5	.06	.63		5	2.00	----------

[1] Less than 0.06 inch.

The possibility that the rapidity of penetration was due to a change in the wood brought about by steaming was investigated. Longitudinally and tangentially matched steamed and unsteamed blocks

[6] VAUGHAN, R. E. A METHOD FOR THE DIFFERENTIAL STAINING OF FUNGOUS AND HOST CELLS. Ann. Mo. Bot. Gard. 1: 241–242. 1914.

[7] Expressed as $\left(\frac{\text{Original weight}-\text{oven-dry weight}}{\text{Oven-dry weight}}\right)100$.

were inoculated with *Trichoderma lignorum*, strain no. 5. The results (tables 2 and 3) show that steaming wood does affect the rate of penetration by *Trichoderma*. The average distance penetrated longitudinally by *Trichoderma* in 20 steamed 2-inch cubes in 60 hours was 1.71 inches (table 2), while the average distance penetrated in 20 matched unsteamed cubes was 0.45 inch.

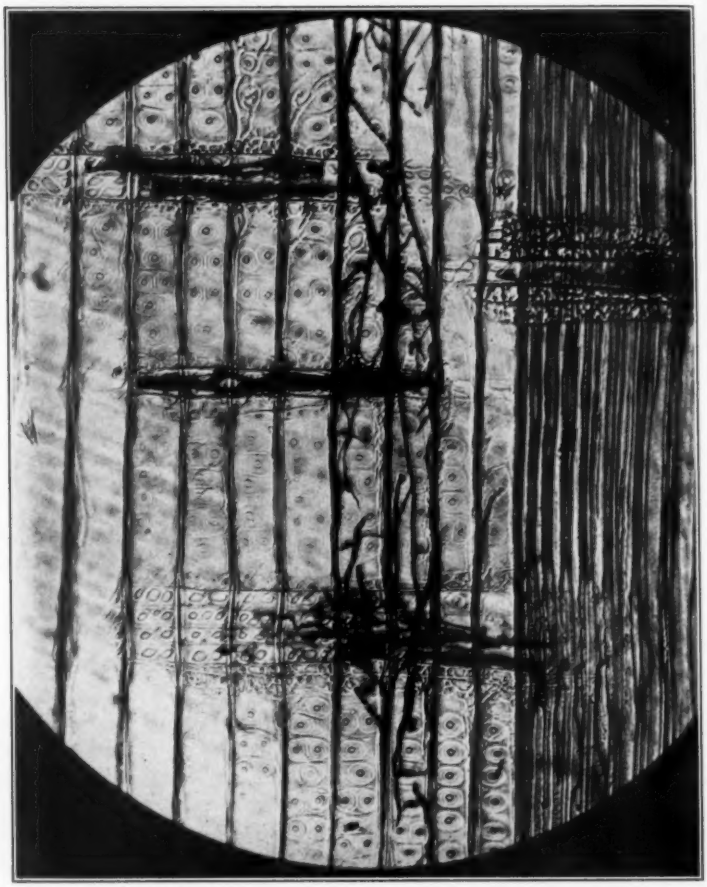

FIGURE 3.—Photomicrograph showing *Trichoderma* mycelium in wood tracheids and ray parenchyma cells of *Pinus taeda* sapwood. × 165.

The relative effect of steaming for 30 minutes at 100° or 121° C. may be seen in table 3. The results indicate that the difference in temperature caused no significant difference in the rate of penetration by *Trichoderma*. The fungus penetrated 1.71 inches into wood steamed at 100°, and 1.63 inches into wood steamed at 121°. During the same period the fungus penetrated only 0.4 inch into the unsteamed wood.

TABLE 2.—*Effect of steaming (at 121° C. for 30 minutes) on the longitudinal penetration of Trichoderma lignorum into sapwood of Pinus taeda in 60 hours*

Unsteamed wood			Steamed wood		
2-inch cube no.	Moisture	Distance penetrated	2-inch cube no.	Moisture	Distance penetrated
	Percent	*Inch*		*Percent*	*Inches*
1	80.8	0.50	2	68.1	1.75
4	97.6	.38	3	77.8	1.88
5	81.3	.44	6	63.7	1.88
8	84.1	.50	7	65.0	1.50
9	82.6	.50	10	64.6	1.75
12	83.4	.56	11	71.0	1.94
13	78.1	.44	14	67.4	1.69
16	94.2	.44	15	72.3	1.94
17	89.5	.56	18	77.8	2.00
20	95.8	.44	19	79.2	1.81
21	86.8	.50	22	67.0	1.88
24	77.0	.38	23	66.6	1.56
25	86.0	.31	26	70.2	1.50
28	90.6	.56	27	66.6	1.81
29	90.4	.50	30	108.2	1.75
32	112.6	.25	31	97.5	1.56
33	95.2	.44	34	77.4	1.75
36	98.9	.50	35	87.7	1.50
37	96.4	.44	38	78.2	1.31
40	106.2	.38	39	79.1	1.50
Average	90.4	.45	Average	75.3	1.71

TABLE 3.—*Effect of steaming (at 100° and 121° C. for 30 minutes) on the longitudinal penetration of Trichoderma lignorum into sapwood of Pinus taeda in 60 hours*

Unsteamed wood			Wood steamed at 100° C.			Wood steamed at 121° C.		
2-inch cube no.	Moisture	Distance penetrated	2-inch cube no.	Moisture	Distance penetrated	2-inch cube no.	Moisture	Distance penetrated
	Percent	*Inch*		*Percent*	*Inches*		*Percent*	*Inches*
1	104.3	0.31	2	100.1	1.69	3	81.9	1.50
5	118.4	.31	6	101.5	1.88	4	93.2	1.38
9	117.3	.44	7	93.1	1.56	8	92.5	1.94
10	111.0	.44	11	101.8	1.75	12	94.9	1.75
14	108.9	.38	15	102.3	2.00	13	88.7	1.50
18	104.6	.44	16	93.7	1.50	17	88.0	1.94
19	107.6	.50	20	97.7	1.94	21	86.2	1.75
23	104.6	.38	24	89.2	1.56	22	83.4	1.38
27	92.4	.38	25	81.3	1.50	26	77.4	1.50
Average	107.7	.40	Average	95.6	1.71	Average	87.4	1.63

Since wood loses moisture during steaming, it was thought that perhaps the steaming might have reduced the moisture of the wood so that the steamed blocks contained only the amount required for the optimum growth of *Trichoderma*, while the unsteamed blocks were too wet. However, the highest moisture content (table 2) of any one steamed block was 108.2 percent, and the lowest moisture content of any steamed block was 63.7 percent; yet the fungus traveled only 0.13 inch farther in the latter. A similar relation existed in the unsteamed blocks. *Trichoderma* grew 0.38 inch into an unsteamed block having a moisture content of 106.2 percent and the same distance into another having a moisture content of 77 percent. The fungus grew very differently on steamed and unsteamed blocks having approximately the same moisture content (table 4).

TABLE 4.—*Depth of penetration of Trichoderma lignorum into steamed (for 30 minutes at 121° C.) and unsteamed blocks of Pinus taeda sapwood having approximately the same moisture content*

Unsteamed blocks		Steamed blocks	
Moisture (percent)	Depth of penetration	Moisture (percent)	Depth of penetration
	Inch		*Inches*
108.9	0.38	108.2	1.75
97.6	.38	97.5	1.56
86.8	.50	86.2	1.73
78.1	.44	78.2	1.31
77	.38	77.4	[1] 1.63

[1] Average for 2 blocks.

SUMMARY

The penetration of *Trichoderma lignorum* into sapwood of *Pinus taeda* was determined by the presence of the fungus in the ray parenchyma cells, ray tracheids, wood tracheids, and resin ducts. The greatest number of hyphae were found in the ray parenchyma and wood tracheids. This was true for both strains used.

The hyphae passed through the natural openings of the wood.

The fungus penetrated the wood both radially and longitudinally, but there was evidence that it grew more rapidly in the longitudinal direction.

There was no significant difference in the rate of penetration between the cellulose-dissolving strain and the noncellulose-dissolving strain.

Trichoderma lignorum penetrated both steamed and unsteamed sapwood; however, steamed wood was penetrated about four times faster than unsteamed wood. Steam sterilization at 100° C. for 30 minutes had the same effect as steam sterilization at 121° for the same length of time, so far as could be determined by the growth of the fungus in the wood.

The difference in moisture content between steamed and unsteamed wood did not appear to be the cause of the difference in the rate of penetration by *Trichoderma lignorum*.

RELATIVE RESISTANCE TO BACTERIAL WILT OF CERTAIN COMMERCIAL AND SELECTED LOTS OF ALFALFA[1]

By J. L. WEIMER, *senior pathologist, Division of Forage Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture,* and B. A. MADSON, *head of Division of Agronomy, California Agricultural Experiment Station*

INTRODUCTION

The United States Department of Agriculture and certain State experiment stations have published a number of papers (*1–13*) [2] giving the results of research seeking an alfalfa (*Medicago sativa* L.) resistant to bacterial wilt (*Phytomonas insidiosa* (L. McC.) Bergey et al.). Westover (*12*) has pointed out that Ladak, Hardistan, Kaw, and the commercial Turkistan alfalfas have shown varying degrees of resistance. All of these alfalfas, however, are of the winter-hardy type, which, regardless of temperature, becomes more or less dormant early in the autumn. Owing to this characteristic, the growth of Turkistan and Ladak alfalfas at Riverside, Calif., is greatly retarded as early as September and is much delayed in the spring. These alfalfas, therefore, are not suitable for commercial production in the Southwest. In view of this fact it seems desirable either to find a selection from one of these alfalfas with the dormant character less pronounced or to search for bacterial wilt resistance in the less winter-hardy types of alfalfa. With this purpose in mind a study was undertaken to compare the relative resistance to bacterial wilt of a number of different lots of alfalfa under California conditions. The present paper is a progress report of the work on this project during the 6 years 1930–35.

METHODS

Most of the seed lots studied were supplied by H. L. Westover, of the Bureau of Plant Industry, United States Department of Agriculture, Washington, D. C. Samples of locally grown seed also were included. The seed was sown in the greenhouse and in rows in the field. The plants usually were 4 to 8 months old when inoculated. The roots were dug carefully, so that a section of the taproot 4 or more inches long remained intact. Besides the broken roots, additional wounds for the entrance of the bacteria were provided by scraping some of the corky layer from the surface of one side of the taproot. This method of wounding the plants was similar to the one described by Jones (*2*). Usually a small segment of the lower end of the taproot was cut off just before inoculation. The plants were inoculated by immersing the roots for several minutes in a heavy bacterial suspension. Bacteria from fresh cultures of a recent isolation were used. All the bacteria that could be scraped and rinsed conveniently from about 25 agar slant cultures were used for

[1] Received for publication Dec. 12, 1935; issued April 1936. Cooperative investigations between the California Agricultural Experiment Station and the Division of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

[2] Reference is made by number (italic) to Literature Cited, p. 555.

each gallon of suspension. The plants usually were set in the ground as soon as possible after inoculation, although in one instance the inoculations were made several days prior to replanting with equally good results.

The plot at Delhi, Calif., where much of the early work was done, was on light sandy soil on which badly wilted plants had grown the previous season, while that at Davis, Calif., used later, was on a heavy black soil. The plants were inoculated either in late autumn or early spring and were allowed to grow undisturbed for several months to a year, more commonly the latter, before they were dug and examined for the presence of bacterial wilt. The taproots were cut off just above the broken end, usually 6 to 12 inches below the crown, and the presence or absence of the characteristic yellowing due to bacterial wilt was determined. Plants which showed no top symptoms of bacterial wilt and whose roots were free of the characteristic yellow color were classed as healthy and the remainder as diseased. In some instances samples of diseased roots were placed in a fixing solution and later studied to confirm the field diagnosis. After the plants were examined those classed as healthy were again wounded, inoculated, and planted and allowed to grow another 6 months or a year. If any plants remained healthy after the second treatment, they were transferred to the greenhouse for seed production. In one case all of the plants in the plot that appeared to be healthy were permitted to go to seed the latter part of the summer following the second inoculation, the tops of the plants showing disease being kept cut to prevent blossoming and crossing with the others. The seed thus obtained was used in an experiment the results of which are shown later. (See table 3.)

EXPERIMENTAL DATA

TESTING UNSELECTED SEED LOTS

The results obtained from inoculating plants of 38 different seed lots of alfalfa are recorded in table 1. As shown in column 3, varying numbers of plants of the different lots were used. These plants were grown in rows in a field at Riverside, Calif., from seed sown March 12, 1930. They were dug October 23 and 24, 1930, inoculated, wrapped carefully in newspaper, packed in boxes and shipped by express to Delhi, Calif., where they were planted on October 27 to 29, 1930. They grew well for a time, but after a few months began dying rapidly from bacterial wilt. The percentage of plants still healthy in October 1931 is given in table 1, column 4.

Table 1, column 5, shows the number of plants that were judged to be worthy of further trial in October 1931. These plants for the most part were healthy, although a few had slight bacterial wilt infections. All of them were reinoculated and replanted as previously described. The results of this second inoculation and the additional year of growth on badly infested soil are evident from the figures in column 6, table 1. Four of the lots were 100-percent dead or diseased by the end of the first year, and many others were only slightly less seriously affected. Twenty of the reinoculated plants were 100-percent affected by the end of the second year. Only an occasional plant had survived and was apparently healthy. It will be noted that greater percentages of

the Turkistan, Hardistan, and Ladak plants were left than of any of the others. A few plants of the less hardy alfalfas, such as Hairy Peruvian and Kansas Common, remained. It is quite evident that a single inoculation did not serve to eliminate all of the susceptible plants. This observation confirms those of Jones (*3*) and Peltier and Tysdal (*8*).

TABLE 1.—*Relative resistance of different seed lots of alfalfa to bacterial wilt, as indicated by percentage of plants uninfected after 1 and 2 inoculations and by percentage of original number of plants still healthy at end of second year (1930–32)*

Variety or source	Accession no. [1]	Plants inoculated October 1930	Plants uninfected October 1931	Plants reinoculated October 1931	Plants uninfected October 1932	Plants uninfected after 2 inoculations
		Number	*Percent*	*Number*	*Percent*	*Percent*
Arabian		161	0.00	0		
India	Comm. 2470	216	.00	0		
Do	Comm. 1724	167	.00	0		
Kansas Common	F. C. 15940	211	.00	0		
Hairy Peruvian	F. C. 15836	221	.45	9	11.11	0.45
Italian	Comm. 2004	213	.47	6	.00	.00
South African	S. P. I. 48094	212	.47	7	.00	.00
Arabian		210	.48	2	.00	.00
Spanish	Comm. 2005	194	.52	5	.00	.00
French	F. P. I. 81489	193	.52	10	.00	.00
Arizona Common	F. C. 15837	183	.55	2	.00	.00
Grimm	N. & R. 444	178	.56	15	.00	.00
South African	F. C. 14204	210	.95	9	.00	.00
Smooth Peruvian	Comm. 2274	203	.99	2	.00	.00
Argentine	Comm. 2346	199	1.01	8	.00	.00
Do	F. C. 15996	193	1.04	2	.00	.00
Hardigan	Comm. 2021	192	1.04	8	12.50	.52
Italian	F. C. 15657	175	1.14	4	.00	.00
California Common (Hemet)		188	1.60	9	.00	.00
Provence	S. P. I. 21195	201	1.99	10	.00	.00
French	F. P. I. 81488	193	2.07	10	.00	.00
Dakota Common	F. C. 16083	221	2.26	21	.00	.00
Kansas Common	N. & R. 435	229	2.62	10	10.00	.44
Argentine	Comm. 04153	198	3.03	9	.00	.00
Bebensee		189	3.17	6	.00	.00
Lebeau	Comm. 2622	217	4.15	16	6.25	.46
Cossack	Comm. 2441	209	4.78	9	.00	.00
Provence	F. C. 15744	225	4.89	12	.00	.00
Hairy Peruvian	N. & R. 437	196	6.63	13	7.69	.51
Turkistan	Comm. 2674	181	7.73	9	11.11	.55
Do	191 [2]	126	9.52	9	22.22	1.59
Do	S. P. I. 31811	129	12.40	16	18.75	2.33
Hardistan		208	18.27	22	13.64	1.44
Ladak		171	20.47	31	16.13	2.92
Turkistan	S. P. I 20988	178	26.97	40	15.00	3.37
Do	F. C. 15754	174	30.46	48	4.17	1.15
Do	Comm. 2230	68	30.88	18	5.56	1.47
Do	F. C. 14786	125	43.20	48	12.50	4.80

[1] F. C. indicates accession numbers of the Division of Forage Crops and Diseases; S. P. I., Division of Seed and Plant Introduction (since changed to F. P. I., Division of Plant Exploration and Introduction); N. & R., New and Rare Field Seed Distribution; Comm., seed purchased from seedsmen.

[2] Accession number of the California Agricultural Experiment Station.

Seed of other lots of alfalfa was sown in rows in the field at Davis, Calif., in April 1931, and the plants were inoculated and planted at Delhi in October 1931. The data obtained from this experiment are recorded in table 2. Here again four of the lots, namely, Morocco F. P. I. 88912 and three Spanish lots, failed to survive the first inoculation. Many others fared little better. Again the Turkistan, Hardistan, and Ladak lots contained more resistant individuals than any of the others except Iran (Persia) F. P. I. 86362. Since most Persian strains show relatively low resistance, the higher degree of resistance

displayed by F. P. I. 86362 raises a question as to whether it came originally from Turkistan.

The relative resistance to bacterial wilt of common alfalfa from Hemet, Riverside County, Calif., and from Modoc County, Calif., so far as known, has not been reported previously. Out of 188 plants of alfalfa from Hemet inoculated in October 1930 (table 1) none survived the 2-year test, while of the 191 plants first inoculated in October 1931 (table 2) only 2.09 percent remained healthy at the end of the second year. A single plant of the alfalfa from Modoc County withstood the double inoculation.

Arizona Common and Hairy Peruvian alfalfas, which are commonly grown in California as well as in neighboring States, proved to be very susceptible in these tests as well as in those previously reported by other workers. Apparently the alfalfa commonly grown in California, regardless of whether the seed comes from Modoc or Riverside Counties or from Arizona, is almost certain to prove susceptible to bacterial wilt.

TABLE 2.—*Relative resistance of different seed lots of alfalfa to bacterial wilt, as indicated by percentage of plants uninfected after 1 and 2 inoculations and by percentage of original number of plants still healthy at end of second year (1931–33)*

Variety or source	Accession no.	Plants inoculated October 1931	Plants uninfected October 1932	Plants reinoculated October 1932	Plants uninfected October 1933	Plants uninfected after 2 inoculations
		Number	*Percent*	*Number*	*Percent*	*Percent*
Morocco	F. P. I. 88912	68	0.00	0	0.00	0.00
Spanish	F. P. I. 89887	200	.00	0	.00	.00
Do	F. P. I. 89886	205	.00	0	.00	.00
Do	F. P. I. 89882	204	.00	0	.00	.00
California Common (Modoc County).		210	.48	1	100.00	.48
French	F. C. 19274	140	.71	1	100.00	.71
Spanish	F. P. I. 89635	203	.99	2	50.00	.49
French	F. C. 19273	154	1.30	2	.00	.00
Kansas Common	F. C. 19180	202	1.98	4	25.00	.49
California Common (Hemet).		191	4.71	9	44.44	2.09
Morocco	F. P. I. 88936	201	2.49	5	40.00	1.00
Spanish	F. P. I. 89884	170	2.94	5	40.00	1.18
Morocco	F. P. I. 88914	33	3.03	1	.00	.00
Spanish	F. P. I. 89846	199	6.03	12	33.33	2.01
Iran (Persia)	F. P. I. 86361	197	8.63	17	58.82	5.08
Hardistan		192	18.75	35	54.29	9.90
Turkistan	F. P. I. 85751	206	20.87	43	46.51	9.70
Ladak	F. C. 15988	94	23.40	22	36.36	8.51
Turkistan	F. C. 19300	206	28.16	58	56.90	16.02
Do	F. C. 19316	209	34.93	71	73.24	24.88
(Iran) Persia	F. P. I. 86362	42	35.71	14	78.57	26.19

TESTING SINGLE-PLANT PROGENY

Data obtained by inoculating the progeny of single plants are given in table 3. Each entry in column 3, combined with the corresponding entries in columns 1 and 2, is the designation of a single plant. These plants include most of those surviving the second inoculation shown in column 7, table 1. As previously stated (p. 548), the seed was obtained during the summer of 1932 by open pollination. This seed was sown in the greenhouse at Davis in the early winter, and the plants were inoculated and transplanted to the field on April 25, 1933. When the plants were examined on November 2, 1933, there were

almost no top symptoms of bacterial wilt and the stand was considered almost perfect. The plants remaining healthy were reinoculated, replanted, and permitted to grow until November 1934. The final results are shown in columns 7 and 8, table 3.

These data are of considerable interest, since they show the reaction to bacterial wilt of plants whose parents withstood a very severe test for resistance. The percentage of healthy plants remaining after the second inoculation varied from 0 to 100, being in general considerably higher than in previous experiments. For example, in table 1 Turkistan F. C. 14786 shows 43.20 and 12.50 percent, respectively, of healthy plants surviving the first and second inoculations. In table 3 is shown the reaction of the progeny of six different plants of Turkistan F. C. 14786. Here the percentage of healthy plants after two inoculations varied from 100 in nos. 4 and 8 to 38.54 in no. 16, the average percentage of the progenies of all six of the plants remaining healthy being 64.57. On the other hand, Turkistan 191–2 after the first inoculation showed the same percentage of healthy plants (22.22) as the original stock when inoculated the second time (table 1, column 6), but no plants in the former survived the second inoculation. Turkistan 191–1 likewise failed to show any significant increase in resistance over its original stock.

TABLE 3.—*Relative resistance of the progeny of individual plants which had survived 2 inoculations*

[The seed was produced by open pollination]

Variety or source	Accession no.	Plant selection no.	Plants inoculated April 1933	Plants uninfected November 1933	Plants reinoculated November 1933	Plants uninfected November 1934	Plants uninfected after 2 inoculations
			Number	*Percent*	*Number*	*Percent*	*Percent*
Turkistan	191	1	61	37.70	22	31.82	11.48
		2	9	22.22	2	.00	.00
	F. C. 14786	4	2	100.00	2	100.00	100.00
		6	25	96.00	22	63.64	56.00
		8	1	100.00	1	100.00	100.00
		10	7	57.14	4	75.00	42.86
		13	30	80.00	21	71.43	50.00
		16	96	77.08	67	55.22	38.54
	F. C. 15754	1	16	87.50	12	83.33	62.50
		5	23	52.17	12	75.00	39.13
	S. P. I. 20988	1	132	52.27	67	43.28	21.97
		10	3	33.33	1	100.00	33.33
		14	38	78.95	26	30.77	21.05
		18	1	.00			
	S. P. I. 31811	1	8	37.50	2	50.00	12.50
		2	32	81.25	25	24.00	18.75
		4	110	66.36	68	55.88	34.55
Ladak		1	37	67.57	24	62.50	40.54
		2	17	47.06	6	83.33	29.41
		3	69	53.62	35	48.57	24.64
		8	26	80.77	20	65.00	50.00
		11	40	47.50	19	52.63	25.00
Kansas Common	N. & R. 435	1	19	47.37	9	11.11	5.26
Lebeau	Comm. 2622	2	190	15.79	29	20.69	3.16
Hardigan	Comm. 2621	1	2	50.00	1	100.00	50.00
Hairy Peruvian	F. C. 15836	1	3	66.67	1	100.00	33.33

Similar comparisons of other plants and their respective progenies, as listed in the tables, show that resistance to bacterial wilt is transmitted to the offspring in different degrees by different resistant plants. In some instances there seems to be no increase in resistance

in the first generation over the seed stock, while the reverse is true in other cases. Obviously the fact that a plant has survived two inoculations and 2 years' growth on infested soil does not necessarily mean that it will produce progeny of a higher degree of resistance. A similar conclusion has been reached by Jones (*2*), Brink, Jones, and Albrecht (*1*), and Peltier and Tysdal (*8*).

In this connection tables 4 to 6 are of interest. Table 4 is designed to show more clearly the comparative resistance of the seed stock and the first generation of certain plants of some seed lots tested. The percentages of plants of each generation remaining healthy after two inoculations are given in columns 4 and 5. In table 4, columns 1 and 2, the first item in each group of plants recorded represents the stock from which the other plants were obtained. For example, the original stock of Turkistan 191, whose resistance is shown in table 1, is compared with the progeny of 191–1 and 191–2, two plants selected from this stock. The original number of plants of 191 set out, as shown in table 1, column 3, was 126. Of these only 2 (191–1 and 191–2) survived the second inoculation, or, in other words, 1.59 percent remained healthy (column 4, table 4). The percentages of the progenies of these two plants that were still healthy after two inoculations are given in column 5, table 4. In like manner Turkistan alfalfas F. C. 14786, F. C. 15754, S. P. I. 20988, S. P. I. 31811, and several other seed stocks are compared with their respective progenies. The increase in resistance is quite striking in some cases and nil in others.

TABLE 4.—*Comparison of the resistance of certain seed lots with that of their progeny*

[Progeny from open-pollinated seed]

Variety or source	Accession no.	Plant selection no.	Percentage of healthy plants after 2 inoculations		Variety or source	Accession no.	Plant selection no.	Percentage of healthy plants after 2 inoculations	
			Original stock	Progeny				Original stock	Progeny
Turkistan	191		1.59		Turkistan	S. P. I. 31811		2.33	
		1		11.48			1		12.50
		2		.00			2		18.75
	F. C. 14786		4.80				4		34.55
		4		100.00				2.92	
		6		56.00			1		40.54
		8		100.00			2		29.41
		10		42.86	Ladak		3		24.64
		13		50.00			8		50.00
		16		38.54			11		25.00
	F. C. 15754		1.15		Kansas Common.	N. & R. 435		.44	
		1		62.50			1		5.26
		5		39.13	Lebeau	Comm. 2622		.46	
			3.37				2		3.16
	S. P. I. 20988	1		21.97	Hardigan	Comm. 2621		.52	
		10		33.33			1		50.00
		14		21.05	Hairy Peruvian	F. C. 15836		.45	
		18		.00			1		33.33

Table 5 gives the reaction to bacterial wilt of progenies of some of the plants shown in table 4, but from seed grown in the greenhouse and hence selfed. The percentages of healthy plants surviving the two inoculations are given in column 8. These figures enable one to compare the relative resistance of the progenies obtained by close pollination (table 5) with those secured by open pollination (tables 3 and 4)

as well as with the original seed stock (table 1). In general, there was considerable increase in resistance in the progenies of these selected plants when close-pollinated over that of the original stock from which the parent plants were selected. The progenies of the parent plants when open-pollinated also showed more or less increase in resistance. However, between the two sets of progenies there was no consistent difference that might be attributed to the difference in the method of pollination.

TABLE 5.—*Resistance to bacterial wilt of progeny of resistant plant selections*

[Progeny from close-pollinated seed]

Variety or source	Accession no.	Plant selection no.	Plants inoculated April 1934	Plants uninfected November 1934	Plants reinoculated November 1934	Plants uninfected May 1935	Plants uninfected after 2 inoculations
			Number	*Percent*	*Number*	*Percent*	*Percent*
Turkistan	F. C. 14786	127	56	50.00	26	92.31	42.86
		128	122	38.52	47	82.98	31.97
		143	13	69.23	8	87.50	53.85
	S. P. I. 20988	131	39	48.72	19	78.95	38.46
		132	64	45.31	27	100.00	42.19
		137	29	24.14	8	37.50	10.34
		139	33	51.52	15	53.33	24.24
		144	20	20.00	4	100.00	20.00
	S. P. I. 31811	138	29	17.24			
	Comm. 2230	141	52	65.38	34	88.24	57.69
	F. C. 15754	126	177	35.03	62	93.55	32.77
		135	71	38.03	28	67.86	26.76
Hardistan			45	51.11	21	66.67	31.11
Ladak		52	55	63.64	33	75.76	45.45
		53	8	50.00	4	75.00	37.50
Hardigan	Comm. 2621	49	73	54.79	39	20.51	10.96
California Common (Hemet).			28	10.71	3	66.67	7.14
Hairy Peruvian (control).			9	22.22	2	.00	.00
Grimm (control)	F. C. 22047		16	.00	0	.00	.00

F. R. Jones contributed some alfalfa plants which he had selected for their resistance to bacterial wilt at Madison, Wis. These were planted in the greenhouse at Davis, Calif., and seed was obtained from most of them. The plants from this seed were inoculated in the usual way. The inoculations were made in April and November 1934, and the plants were examined in November 1934 and May 1935, respectively. The percentage of the progenies from each plant remaining healthy is given in table 6. The source of the parent plants is shown in table 6, column 1. These plants were presumably of the Common variety, 1301–1, 1304–8, and F. C. 15749 being recorded by Jones as of the semihardy type. Some of these plants produced progeny of a low degree of resistance, as shown in column 8, while the reverse is true of others. A better understanding of the potential value of a line in the nonwinter-hardy group, which shows 50 percent or more of resistant plants, is obtained by a comparison with those of the same group; for example, with the California Common grown in the vicinity of Hemet and Hairy Peruvian, listed in table 5, and with other nonhardy alfalfas, listed in tables 1 and 2. Very commonly the plants belonging to the nonhardy group are 100-percent affected as a result of the first inoculation. Several of the plants sent by Jones, some of which are nonhardy, have produced progeny apparently equal in

resistance to the Turkistans and Ladak. The prospect of obtaining some valuable selections from these plants seems promising.

TABLE 6.—*Resistance of the progeny of plants selected by F. R. Jones at Madison, Wis.*

[Selfed seed grown at Davis, Calif.]

Variety and source of seed lot from which selections were made	Accession no.	Plant selection no.	Plants inoculated April 1934	Plants uninfected November 1934	Plants reinoculated November 1934	Plants uninfected May 1935	Plants uninfected after 2 inoculations
			Number	*Percent*	*Number*	*Percent*	*Percent*
Kansas Common	1225 [1]	----	124	21.77	25	28.00	5.65
		48	55	12.73	7	42.86	5.45
		101	46	30.43	13	100.00	28.26
		102	74	56.76	41	73.17	40.54
		111	22	50.00	11	81.82	40.91
Montana Common	1300 [1]	3-108	89	21.35	18	66.67	13.48
		3-109	60	75.00	39	79.49	51.67
		3-110	30	56.67	17	76.47	43.33
Kansas Common	1301 [1]	1-39	124	16.94	20	50.00	8.06
	1302 [1]	2-121	45	48.89	20	80.00	35.56
		2-105	37	62.16	20	100.00	54.05
		2-107	100	76.00	69	85.51	59.00
		2-116	21	71.43	14	100.00	66.67
	1303 [1]	2-117	25	72.00	15	100.00	60.00
		2-120	26	53.85	14	100.00	53.85
		2-122	59	40.68	21	80.95	28.81
		2-124	77	76.62	58	74.14	55.84
		3-118	19	78.95	15	73.33	57.89
		2-47	21	28.57	7	57.14	19.05
		2-103	18	11.11	2	50.00	5.56
	1304 [1]	2-106	37	5.41	2	100.00	5.41
		8-13	103	29.13	28	42.86	11.65
		8-20	36	27.78	15	93.33	38.89
Spain	F. P. I. 88141	30	75	52.00	38	97.37	49.33
	F. P. I. 88143	56	16	50.00	8	100.00	50.00
	F. P. I. 89643	(1)-50	22	9.09	2	50.00	4.55
	F. P. I. 89631	55	23	.00	----	----	.00
	F. P. I. 89883	54	3	.00	----	----	.00
	F. P. I. 89854	2-43R	115	21.74	24	62.50	13.04
		25	96	35.42	33	48.48	16.67
Portugal	F. P. I. 89904	3	82	53.66	42	54.76	28.05
		5	91	42.86	38	47.37	19.78
		11	26	19.23	4	100.00	15.38
		14	93	55.91	52	50.00	27.96
	F. P. I. 89906	(1)-22R	76	17.11	13	76.92	13.16
Morocco	F. P. I. 89973	51	17	35.29	4	.00	.00
		AC110-26	100	19.00	19	68.42	13.00
Kansas Common	F. C. 15749	34	68	64.71	43	79.07	50.00
Hairy Peruvian (control)	----	----	9	22.22	2	.00	.00
Grimm (control)	F. C. 22047	----	16	.00	0	.00	.00

[1] Accession number of Wisconsin Agricultural Experiment Station

SUMMARY

Alfalfa plants produced from 59 different lots of seed from various sources have been tested for resistance to bacterial wilt at Delhi and Davis, Calif.

The plants were inoculated twice and grown in field plots for 6 months to 1 year following each inoculation. Seed was obtained from the plants which remained healthy and the resistance of the new generation was tested.

The results confirm those of other workers in that they show that Turkistan, Hardistan, and Ladak alfalfa seed lots contain the highest percentages of resistant individuals. A few plants of some of the other seed lots survived the two inoculations. The progenies of these are being tested.

Iran (Persia) F. P. I. 86361 and F. P. I. 86362 contained more resistant individuals than most of the others, with the exception of Turkistan, Hardistan, and Ladak, and, since most lots from Iran have shown relatively low resistance, the question arises as to whether these lots came originally from Turkistan.

For the most part, progenies of plants that survived two inoculations gave a somewhat higher percentage of resistant individuals than did the original stock. Some of the progenies were no more resistant than the stock from which the original selections were made, while the reverse was true in other cases, showing that resistance is transmitted to the offspring in varying degrees by different plants.

Plants from 38 different individuals selected by F. R. Jones, many of which were from the nonhardy group of alfalfas, were tested. The progenies of some of these plants contained many individuals which survived two inoculations.

LITERATURE CITED

(1) BRINK, R. A., JONES, F. R., and ALBRECHT, H. R.
1934. GENETICS OF RESISTANCE TO BACTERIAL WILT IN ALFALFA. Jour. Agr. Research 49: 635–642, illus.

(2) JONES, F. R.
1930. BACTERIAL WILT OF ALFALFA. Jour. Amer. Soc. Agron. 22: 568–572.

(3) ———
1934. TESTING ALFALFA FOR RESISTANCE TO BACTERIAL WILT. Jour. Agr. Research 48: 1085–1098.

(4) KIESSELBACH, T. A., ANDERSON, A., and PELTIER, G. L.
1930. A NEW VARIETY OF ALFALFA. Jour. Amer. Soc. Agron. 22: 189–190.

(5) PELTIER, G. L.
1933. THE RELATIVE SUSCEPTIBILITY OF ALFALFAS TO WILT. Nebr. Agr. Expt. Sta. Research Bull. 66, 16 pp.

(6) ———and JENSEN, J. H.
1930. ALFALFA WILT IN NEBRASKA. Nebr. Agr. Expt. Sta. Bull. 240, 35 pp., illus.

(7) ——— and TYSDAL, H. M.
1930. THE RELATIVE SUSCEPTIBILITY OF ALFALFAS TO WILT AND COLD. Nebr. Agr. Expt. Sta. Research Bull. 52, 15 pp.

(8) ——— and TYSDAL, H. M.
1934. WILT AND COLD RESISTANCE OF SELF-FERTILIZED LINES OF ALFALFA. Nebr. Agr. Expt. Sta. Research Bull. 76, 26 pp., illus.

(9) SALMON, S. C.
1930. THE REACTION OF ALFALFA VARIETIES TO BACTERIAL WILT. Jour. Amer. Soc. Agron. 22: 802–810.

(10) TYSDAL, H. M.
1935. ALFALFA WILT CONTROL BY BREEDING MAKING REMARKABLE PROGRESS. U. S. Dept. Agr. Yearbook 1935: 119–120, illus.

(11) WESTOVER, H. L.
1928. ALFALFA OF LADAK VARIETY IS PROMISING FOR NORTHERN STATES. U. S. Dept. Agr. Yearbook 1927: 94–95, illus.

(12) ———
1933. ALFALFA LOSSES FROM BACTERIAL WILT HEAVY; RESISTANT KIND SOUGHT. U. S. Dept. Agr. Yearbook 1933: 212–216.

(13) WILKINS, F. S., and WESTOVER, H. L.
1934. TURKESTAN ALFALFA AS COMPARED WITH GRIMM FOR WILT-INFECTED SOILS IN IOWA. Jour. Amer. Soc. Agron. 26: 213–222.

U. S. GOVERNMENT PRINTING OFFICE: 1936